THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

FEB 18 1918

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VOL. XLV—NO. 3

Issued February 1, 1918

BALTIMORE, U. S. A.

1918

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From two to three volumes each of about six hundred pages are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries $5.25.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, Johns Hopkins Medical School, Washington and Monument Streets, Baltimore, Md. They should be packed flat, carefully protected from injury and insured.

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 45 FEBRUARY 1, 1918 No. 3

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 80, AND FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, NO. 305

THE PHYSIOLOGY OF ASCIDIA ATRA LEUSEUR

III. The Blood System

SELIG HECHT

Received for publication November 6, 1917

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I. INTRODUCTION

Ascidia atra is a large tunicate possessing an opaque, blue-black test. It is common in the Bermuda Islands and may be collected in sufficient numbers for experimentation in the immediate vicinity of Agar's Island.

In the previous papers of this series (Hecht, '18 a and '18 b), and in an earlier paper (Hecht, '16), the activities of this species have been described. The reader is referred to these accounts of the general and sensory physiology for the more important aspects of the life of Ascidia.

The vascular system of Ascidia atra consists essentially of an arrangement of continuous cavities within which the blood is kept in constant movement by the contractions of a muscular heart. By means of this shifting of the blood, the respiratory, nutritive and secretory products, which are produced by the activities of different portions of the organism, are made available for their general and local utilization. As a result, any adequate consideration of the physiologic relations of the vascular supply would mean a treatment of almost the complete physiology of the animal. To avoid this I propose, therefore, to treat the blood system merely as an organic mechanism by itself, and to describe as far as possible the physiology of the tissues and organs which compose it.

II. BLOOD

1. Path of blood stream. The cavities through which the blood flows represent those remains of the primitive blastocoel of the embryo which have not become filled with connective tissue strands and jelly. In the large monascidians, to which A. atra belongs, the connective-tissue cells near the blood spaces arrange themselves so as to form tubes with fairly definite walls (Seeliger '93–11, p. 531). It is through these that the vascular fluid moves. The vessels of the test are formed by the evagination of the ectoderm into the body of the test.

In spite of this apparently indefinite mode of origin, the blood channels of the adult present quite a uniform development, and in Ascidia their pattern and direction show individual variations only in the smaller vessels. There are present three large and well-defined vessels which determine the major distribution of the blood to the body. Two of these may best be described in their relation to the heart; the third in relation to the branchial sac. Examination of figure 1 will aid in an understanding of the following description of the path of the blood stream.

The heart is a tubular organ which lies on the ventral side of the animal, and extends for nearly two-thirds of its length. From its anterior or branchial end there arises the hypobranchial vessel. This is the great ventral channel which lies below the endostyle and through it the blood from the heart goes to the branchial sac. Before breaking up

into its smaller divisions, which communicate directly with the transverse vessels of the branchial sac, the hypobranchial vessel gives off a branch. This and an accompanying vessel (stippled in the figure) from the branchial sac alternately furnish the blood to the test. The double vessel thus formed constitutes the sole vascular connection between the body of Ascidia and its test.

From the posterior or visceral end of the heart is given off the visceral vessel. This at once breaks up into several branches which lead to the intestine, gonads and other body organs. From these regions the blood is collected by means of small channels into larger vessels, two of which are usually clearly visible on the left side of the body.

The blood from these two paths is led into the dorsal vessel. This is a spacious channel situated dorsally and like the hypobranchial vessel it gives off numerous branches into the transverse vessels of the branchial sac.

The blood which moves in the transverse vessels is in osmotic contact with the seawater which the cilia of the stigmata are constantly driving through the branchial sac. Here the respiratory exchange is accomplished and here most probably occurs the absorption of the organic (Pütter, '07) and inorganic constituents of the seawater. When the heart is beating from the branchial toward the visceral end, the blood from the branchial sac, aerated and containing perhaps other absorbed constituents, is pumped to the visceral regions. Here its oxygen supply is utilized and it becomes charged with the digested products of the food, with the secretions of the nearby glands and with carbon dioxide.

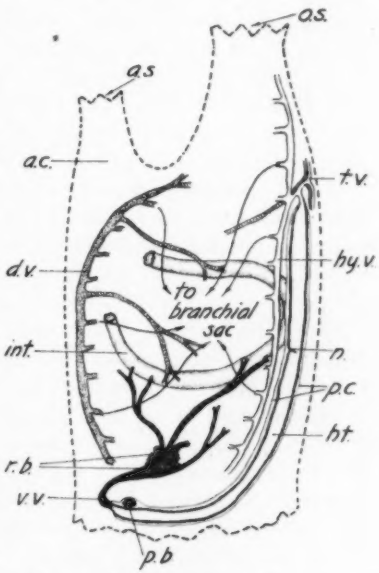

Fig. 1. Semi-diagrammatic representation of the heart and major blood vessels of Ascidia atra viewed from the right side; *a.c.*, atrial cavity; *a.s.*, atrial siphon; *d.v.*, dorsal vessel; *ht.*, heart; *hy.v.*, hypobranchial vessel; *int.*, intestine; *n.*, node; *o.s.*, oral siphon; *p.b.*, pericardial body; *p.c.*, pericardium; *r.b.*, renal body; *t.v.*, test vessel; *v.v.*, visceral vessel.

The blood is thence collected into the dorsal vessel to be further driven into the branchial sac, where the cycle begins all over again.

A phenomenon unique in tunicates is the reversal of the direction of the circulation. When this occurs in Ascidia atra so that the heart is beating from the visceral to the branchial end, it is the dorsal vessel which brings the aerated blood to the viscera. From here it flows through the visceral vessel to the heart and then to the branchial sac by way of the hypobranchial vessel. After being again aerated it is sent to the body through the dorsal vessel.

2. Plasma and cells. The blood of the intact animal is not directly subject to observation, because of the opacity of the test. However, the removal of a portion of the test is readily accomplished and apparently leaves unaffected the normal activities of the organism. The heart is an easily accessible portion of the circulatory system and being transparent, it offers a convenient location for the examination of the blood stream. By carefully slicing away the right ventral face of the test, the heart may be exposed for almost its entire length.

Such a preparation, freshly made, shows the blood as a vivid green, turbid fluid flowing through the heart and nearby vessels. This appearance, however, is entirely misleading. If the animal be allowed to remain undisturbed for a few minutes, the color and turbidity disappear, leaving the blood transparent and colorless. The conditions which cause the green and cloudy appearance will be considered subsequently. At present, however, it must be emphasized that the colorless state of the blood and its transparency are the normal characteristics of the animals of this species. They seem to be the general properties of tunicate blood (Herdman, '04).

The turgidity of the heart in an exposed preparation indicates that the blood is under an appreciable pressure. A similar interpretation is to be placed on the phenomena which are associated with a rupture of the heart or of a major vessel. Under favorable circumstances, a small puncture in the heart of an animal under water results in the blood being forced out to a distance of several centimeters. This appearance is striking because of the rapid color change which the blood undergoes as it leaves the point of injury: a streak of bright green marks the discharged stream.

The blood of Ascidians is distinguishable from that of Appendicularians by the presence of definite, regularly occurring blood-cells (Seeliger '93–11, p. 553). The medium in which these corpuscles are suspended and carried throughout the body is a balanced salt solution containing

protein to a small extent (Henze, '11; Cuénot, '91) and probably other organic substances. In Ascidia atra this plasma is colorless under all experimental conditions. The osmotic pressure of the blood fluid of Phallusia mammillata has been determined by Henze ('11), and it is practically isotonic with seawater: for the plasma $\Delta = 2.07°$, while for seawater $\Delta = 2.12°$. A similar relation undoubtedly exists in Ascidia atra because the blood cells will live and remain active in seawater for several days. Ascidians are thus "poikilosmotic" animals (Höber, '14, p. 37)—marine organisms the osmotic pressure of whose body fluids is isotonic with the seawater in which they live.

A piece of blue litmus paper inserted into a cut in the test of Ascidia rapidly changes to red. The same is true if a piece of the branchial sac or any other tissue containing blood be touched with blue litmus paper. Such a condition was observed for other Ascidians by Henze ('11) and he at first concluded that the blood was acid in reaction. Further study proved this incorrect. If freshly drawn blood of Ascidia be centrifuged, the clear plasma does not turn blue litmus red. It has the same reaction as seawater (cf. Henze, '12). The centrifuged corpuscles, however, are decidedly acid and turn litmus accordingly. In Phallusia the acid was identified by Henze as H_2SO_4. From his data I calculate a concentration of ca. 0.4 N H_2SO_4 within the blood cells. The small volume of blood obtainable from A. atra precluded a quantitative examination. The corpuscles, however, give an unforgotten sour taste.

An effective demonstration of the localization of the acid of the blood may be made by comparing the effect produced by a drop of dilute acid and by a drop of blood on a dry piece of blue litmus paper. The acid spreads out to form a circle several times the diameter of the original drop. As shown in figure 2, it is only at the periphery of the circle that the acid (HCl) fails to be adsorbed to the same extent as the water. The result is a moist circle of red surrounded by a narrow band of moist blue. A drop of blood produces an entirely different appearance. The plasma spreads and leaves a circle of moist blue paper. The corpuscles, however, remain within the confines of the original drop, and produce a small, intensely red space within the much larger one of the adsorbed plasma (cf. fig. 2).

The plasma is therefore alkaline, the corpuscles acid to litmus.

The acidity of the blood cells is of interest with regard to the theory of vital staining. On the assumption that basic dyes penetrate living cells because of the ordinarily alkaline reaction of protoplasm (Höber, '14, p. 432), acid dyes should penetrate easily the acid cells of the blood.

In a preliminary statement Bethe ('14) announced that such is the case. Using many acid stains he found that they entered Ascidian blood cells with great facility. Basic dyes, however, penetrated only feebly.

The blood cells of tunicates are mesenchyme cells and correspond more to the lymphatic cells than to the red corpuscles of vertebrate blood. In Ascidia mentula, Cuénot ('91) described four kinds of amebocytic cells, all of which are more or less related genetically. Like the blood cells of A. fumigata (Heller, '75), however, those of A. atra may best be divided into two classes: unpigmented and pigmented.

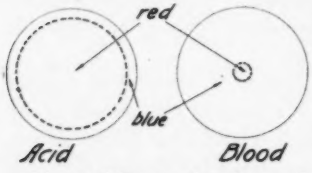

Fig. 2. Effects of a drop of acid (HCl) and of a drop of Ascidia blood on a dry piece of blue litmus paper.

The unpigmented cells are of two kinds at least. The first kind readily assumes an ameboid appearance (*c*, fig. 3) and continually changes shape when observed with the microscope. These cells are all nearly the same size, are quite numerous and, in the living condition, possess an apparently homogeneous protoplasm.

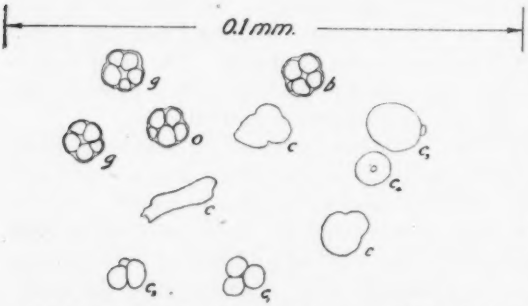

Fig. 3. Blood cells; *b*, blue cells; *c*, ameboid colorless cells; c_1–c_4 colorless cells of constant form; *g*, green cells; *o*, orange cells.

The second type of colorless blood cell retains its spherical form under the conditions of the examination. Its size is variable; often two or three are closely associated (c_1 to c_4). Undoubtedly further cytologic study would show that this type is composed of several classes of cells.

The pigmented corpuscles all appear to be alike in structure but they differ markedly in color and distribution. They are capable of a slight though definite ameboid movement. The most abundant of the pigmented cells, and indeed the most common of all the blood cells, are the green corpuscles (*g*), which occur in all parts of the circulatory system.

Their protoplasm is packed full of a number of spherical granules which are of a bright green color. According to Henze ('13) they are the cells which contain the acid of the blood; with methyl red they stained a deep red, whereas the plasma and seawater became yellow. This signifies a hydrogen-ion concentration of $10^{-4.2}$ to $10^{-6.3}$N within the cell.

The orange cells (*o*) are similar to the green cells in structure but the granules are of a rich orange color. As has already been observed in A. mentula (Cuénot, '91), these orange cells are distributed locally. They are very common in the branchial sac; in some of the papillae I have counted even more orange than green cells. But they are only rarely encountered in the general circulation. Blood drawn from the heart contains only an occasional orange corpuscle, frequently none at all.

The third type of colored cell has a deep blue color (*b*). Whereas the other pigmented cells are transparent, these are quite opaque. The blue cells are distributed in a manner complementary to the orange cells. They are not very common but in blood drawn from the heart they may be easily found. I have, however, seen but very few blue corpuscles in the branchial sac. Genetically they are related to the green cells, of which they probably represent the later stages of existence. It has been possible to observe the change from green to blue in the individual cells of drawn blood.

The localization of the blue and orange blood cells is of importance for an understanding of the functions of the blood and the blood cells. Cuénot concluded that the orange cells of A. mentula do not move with the blood stream. Although my observations on A. atra are not wholly in accord with the idea of this keen observer, the data are too meager for me to venture even a tentative hypothesis. However, the properties of the pigments of the blood cells may furnish a clue to the significance of their localization.

3. Pigment. Respiration is intimately connected with the movements and composition of the blood. The walls of the branchial sac are no more than a complex mass of interwoven blood vessels which, in this way, permit an exchange between the blood and the seawater passing through the sac. The respiratory activity of several tunicates has been measured and it was found that they maintain a definite, though small respiratory exchange (Pütter, '07; Vernon, '95).

The first investigator to study the relation of the blood to the respiration of Ascidians was Harless ('47). He reported that blood freshly drawn from "Ascidia mamillaris" (probably Phallusia mamillata) is a

"*wasserhelle Flüssigkeit,*" which turns blue after remaining exposed to the air for a few minutes. Fresh blood does not change color on contact with oxygen or nitrogen; carbon dioxide, however, at once turns it blue. Oxygen, bubbled through the blue blood makes it colorless, but not as colorless as it was originally. Essentially the same observations were reported by Krukenberg ('80 a), except that he denied the re-formation of colorless from blue blood by the addition of oxygen. It was Krukenberg who first noted that the pigment changes were localized in the cells of the blood.

Apparently the most spectacular contribution to the physiology of Ascidians is the reported presence of a colorless respiratory protein, γ-achroglobine, in the blood of Ascidia, Molgula and Cynthia. Griffiths ('92), its discoverer, assigned it a definite formula and stated that in its reduced state 100 grams would absorb 149 cc. of oxygen at standard pressure and temperature. It will be observed that this binding capacity is greater even than that of haemoglobin.

Using the species employed by the previous investigators, Winterstein ('09) failed to confirm their statements of the color changes and respiratory activities of the blood. He found that the blood of Ascidia certainly turns blue-black on standing, but it does so only after many hours. Moreover, CO_2 has no effect on the color change. Henze ('11) gives corroborative evidence, and my observations of Ascidia atra are also in complete accord with Winterstein's. Often the blood of A. atra requires more than a day to change completely to blue-black.

Winterstein determined the binding power of Phallusia blood, using a modern technic. One hundred cubic centimeters of blood saturated with air contains 0.24 cc. of CO_2, 0.38 cc. of O_2 and 1.33 cc. of N_2. This shows an absorption of these gases by the blood almost identical with their solubility in seawater. The small oxygen binding capacity is evidently a general characteristic of invertebrate blood (Winterstein, '09). It must therefore be concluded that, like the haemocyanin of Limulus (Alsberg and Clark, '14), the blood pigments of Ascidia have a function other than that of absorbing oxygen and carbon dioxide, or perhaps that their method of doing so is different from that of haemoglobin.

The pigment of Ascidian blood is exceptional in its possession of a remarkable chemical element. Henze ('11) describes the chromogen of the blood cells of Phallusia as a protein in combination with the rare element vanadium. The green blood cells of Ascidia atra furnish a pigment which is also a compound containing vanadium. The mass of dried and ignited blood cells, fused with KNO_3 and $NaCO_3$ forms an

alkali vanadate which gives the characteristic color reactions denoting the presence of vanadium.[1]

Chemically, the occurrence of vanadium and its compounds in the blood is important because they possess marked catalytic properties. For example, the oxidation of anilin hydrochloride to anilin black is catalyzed by the presence of one part of V_2O_5 to 100,000 parts of anilin (Witz,'76). Probably it is in the *rôle* of catalyst that the blood pigment is of service in the respiratory activity of ascidians

This element is significant, moreover, from the aspect of its physical properties. Ascidians possess a great variety of colors and color changes in the blood and tissues. Vanadium exists in five stages of oxidation, each showing a series of colors among its compounds. The oxides, for example, are V_2O, V_2O_2 and V_2O_3 which are basic, and V_2O_4 and V_2O_5 which are acid. V_2O_5 forms orange-red crystals, a yellow solution in water and a red soultion with acids and hydrogen peroxide. V_2O_4 is blue, its hydroxide, $V_2O_2(OH)_2$ is brown in alkaline solution. V_2O_3 is green. Such examples may be multiplied. Combinations with organic substances would give these compounds a still greater range of color.

Very probably the green pigment of the blood cells of Ascidia contains vanadium in the stage of oxidation corresponding to V_2O_3 (cf. especially, Henze, '12). That it is present in the green cells is undoubted. A water extract of the pigment turns black on the addition of osmic acid due to the precipitation of a lower oxide of osmium The careful addition of osmic acid to a blood preparation on a slide results in the blackening of the green cells.[2] If the orange cells contain vanadium in its pentavalent state, it is clear why they are not blackened by osmic acid.

4. Coagulation. The clotting of the blood of A. atra is solely an agglutination of the corpuscles,—the so-called first coagulation of invertebrate blood (L. Loeb, '10). The second coagulation, that is, the second-

[1] An example is the following. The fused mass is treated with water. This dissolves the vanadate, giving a colorless solution. To such a solution is then added a crystal of tartaric acid; the solution turns yellow-red at once. On boiling, the color changes to blue, due to the reduction to V_2O_4. Another color test is to acidify the alkali vanadate, and then to add hydrogen peroxide. The solution turns a rich orange color.

[2] The notion once prevalent among cytologists that the blackening of a cell by osmic acid invariably indicates the presence of fat, has led to confusion here as well as elsewhere. Even so careful a student as Cuénot ('91), observing the blackening of the green blood cells of Ascidians, concluded that they were cells which contained a reserve store of fat. This error has been perpetuated in the literature (Von Fürth, '03, '99).

ary clotting of the plasma, occurring in Limulus, for example (L. Loeb, '10), is not encountered in ascidian blood. The cells, pigmented and unpigmented, stick together to form sheets and threads. The strings of clotted blood indicate their composition by their vivid green color. Viewed with the microscope they show the preponderating presence of innumerable green blood cells, which change their form but slightly. The unpigmented cells maintain a slow and continued ameboid activity.

The blood cells will agglutinate on contact with seawater. It should however be pointed out that in a puncture of the circulatory system the blood is already clotted *just before* it leaves the vessels. Moreover, the blood can be made to coagulate within the closed and apparently uninjured blood system. The surface change which makes the cells stick to one another may therefore come about not only through contact with a foreign body (Drew, '10), but also by means of a change in the composition of the plasma.

In a consideration of the factors of coagulation, the behavior of the blood cells outside of the body is very suggestive. The corpuscles may be washed in seawater and stirred up in a watch glass. They then settle and form a thin layer on the bottom of the dish. In several hours the movements of the cells have resulted in the formation of isolated balls of cells a few millimeters in diameter. They remain in this condition for a day or two and then begin to flatten out into discs having thin, irregular margins. The cells may continue alive as long as a week; in my experiments they have always died from neglect. Most of the cells retain a green color, but of a darker shade than in the fresh condition. After the discs have flattened out, there is present in the middle of the plate a little peak of blue cells. Although chiefly located in this central mound, many blue cells are still to be found throughout the mass and especially on the surface of the disc.

I have not observed the formation of these cell masses with sufficient care to be certain whether the coalescence of the cells is wholly fortuitous or not. The entire process, however, possesses a striking resemblance to the formation of restitution masses by the isolated tissue cells of sponges and hydroids (Wilson, '11). The further similarity in the behavior of the smaller groups of blood cells to the appearances which Roux ('96) has called "Cytotaxis," suggests that the agglutination of the blood cells may involve something more than an accidental collision of cells whose surfaces have become sticky.

There remains to be considered still another aspect of the coagulation of the blood. At the beginning of the description of the blood it was

stated that a freshly exposed preparation shows the blood in the heart and vessels to be a vivid green, turbid fluid. This appearance is due to a clotting of the blood within the closed circulatory system due to the mechanical stimulation incident to the operation. After fifteen or twenty minutes, if the animal has been undisturbed, all traces of this green turbidity have disappeared and the blood has become so transparent and colorless that the heart is visible only when it contracts. The green, turbid condition of the blood may be induced again by merely handling the individual roughly: squeezing or bending it for a few seconds. After being replaced into seawater, the heart begins to beat in a jerky manner. This indicates an obstruction due to the clotting within the capillaries and smaller vessels, following which the blood in the heart turns green and turbid.

The maximum of turbidity and color is reached within a minute or two. After a short period, depending on the intensity and duration of the rough handling, the blood begins to revert to the normal condition. The disturbed state of the animals and the slow recovery which follow their collection and transportation to the laboratory are probably due to the effects of a prolonged clotting within the circulatory system.

In demonstrating this internal clotting to my associates in the laboratory, I soon found that the same animal could not be employed for more than four or five times in immediate succession. At the end of such a period the color and turbidity produced by rough handling were slightly different from the normal appearance.

An explanation of this may be that the clotting is caused by the secretion of a substance into the blood. The failure to coagulate after a few stimulations might then be accounted for by the rapid exhaustion of the secretory activity. It is necessary only to call attention to the well studied instance of adrenalin secretion (Cannon, '15) and its effects on body conditions and on blood coagulation, to see the possibilities of an extended investigation of this phenomenon in Ascidia.

III. HEART

The observations on the blood stream of Ascidia atra were made on animals the tests of which had been removed in the region of the heart. The heart itself may be readily exposed for its entire length by the removal of a narrow strip of the test. As long as care is taken not to cut the nearby double blood vessel which goes to the test, such a preparation involves almost no loss of blood and behaves in all other respects normally

1. Structure. The cardiac apparatus of ascidians consists of a double walled tube, the inner wall of which is formed in the embryo by an invagination along the entire extent of the outer wall. The two margins of the invagination have completely fused in A. atra, and the suture is an evident structure of the adult heart. The outer wall remains to form the pericardium, while the inner wall becomes the myocardium or heart proper.

The position of the heart is shown in figure 1. About two-thirds of it lies in an almost straight line along the ventral edge of the right side of the body, with the branchial end well anterior. An abrupt turn in the posterior region of the heart carries the remaining one-third along the posterior edge of the left side, the visceral end terminating close to the dorsal edge of the body. The heart is comparatively long. In a medium sized specimen of about 90 grams body weight, it is 4 or 5 cm. in length. Larger individuals may have a heart as long as 6 or even 7 cm.

Approximately in the middle of that portion of the heart which lies on the right side there is to be observed a definite, though slight constriction. A node similar to this has been described in the hearts of other tunicates, especially in Salpa (e.g., Schultze, '01, p. 250). Unlike the one in Salpa, however, the constriction in Ascidia atra is not the result of an arrested muscular contraction of the quiescent heart. It is always present, whether there is a contraction wave passing over the heart or not. Nor is it caused, as Nicolai ('08, p. 94) supposes, by the bending of a thin-walled, fluid-filled tube. In A. atra the heart makes no bend here; and where it does make a sharp bend toward the dorsal edge no such node is produced. Moreover, the pericardium is also a fluid-filled tube having even a much thinner wall and running exactly parallel to the heart. It should therefore also show a constriction, if Nicolai's idea were correct. But such is not the case. The heart node must consequently be regarded as an anatomically significant structure.

The node shows its presence physiologically as well as structurally. In Ascidia it may well be due to a shifting ventrally of the suture between the pericardium and the heart. An evidence of this is the behavior of the beat in its passage along the heart. This contraction wave is a constriction which runs almost completely around the circumference of the heart, but does not include the suture. In some animals it is easy to see the suture on the dorsal side unaffected as the contraction wave passes by. In such hearts, beating from the visceral toward the branchial end, the wave of constriction is clearly ventral as far as the node.

There it suddenly shifts, and the constriction wave may pass along the right side of the heart or it may even shift until it is visible on the dorsal side.

Another indication of the physiologic presence of the node is a change which may occasionally be observed in the velocity of the contraction wave as it passes the node. In instances of this nature the velocity of the beat in its passage from the visceral end to the node is noticeably greater than from the node to the branchial end.

The influence of the heart node is evidenced in still another way in animals which have suffered a loss of blood. In such a heart, beating from the visceral to the branchial end, the pre-nodal portion may continue to remain contracted as the wave progresses. When, however, the wave has passed the node, the pre-nodal region assumes the relaxed condition and the remainder of the wave is perfectly normal.

It seems quite clear therefore that the node is a real landmark in the structure of the heart of Ascidia atra. Of its functional significance, however, I am in complete ignorance.

The space between the pericardium and the heart—the pericardial cavity—is filled with a transparent fluid which contains, besides a protein substance in solution (Cuénot, '91), a variable quantity of cellular elements. According to Fernandez ('06) these elements are derived from the blood cells, and in the young of several species of ascidians they undoubtedly resemble blood cells closely.

In the pericardial cavity of A. atra there is present, in addition to what has already been mentioned, a grayish, spherical mass which, though unattached, is almost always found at the visceral end of the heart. This localization is due to a slight enlargement of the cavity at this end, into which this pericardial body fits snugly. Occasionally it is dislodged by the contraction of the heart and jerked into the general region of the node, where it is moved now this way, now that, depending on the direction of the heart beat. All the individuals which I examined contained this body in the pericardium. It therefore agrees in its position with the mass described by Fernandez ('06) in the pericardium of A. mentula and A. fumigata, and not with the alleged situation within the heart proper as stated by Heller ('75) for these species.

The size of the pericardial body of A. atra varies with the size of the individual. In animals 3 cm. long it is between 1 and 2 mm. in diameter, while it may reach a diameter of 4 mm. or more in the larger animals. It is composed of concentric layers of material which can be taken off in shreds similar to the layers of an onion. Fernandez ('06) has studied

the pericardial mass of several species of ascidians and finds that it is built up by the continuous deposition of the elements found in the pericardial fluid. To these are added the broken remains of the muscle cells of the heart. The presence of the latter furnishes the explanation (Fernandez,'06) of the occurrence of metamorphosed blood cells in the pericardial cavity and in the pericardial body. A tiny rupture of the wall of the heart results in the passage of a few blood cells, together with the torn muscular region, into the pericardial cavity. This idea is more in harmony with the histological findings than that of van Gaver and Stephen ('07), who concluded that the formation of the pericardial body was the result of the activity of a specific sporozoan parasite.

The tunicate heart proper is described as a single layer of cells the inner ends of which have developed muscle fibrils (Herdman, '04, p. 49). Although Roule ('84) has figured the presence of a delicate endothelial layer on the inner face of the heart of Ciona, Seeliger ('93–11, p. 515) has failed to confirm its existence there. However, Hunter ('02) reported its occurence in Molgula, and Schultze ('10) in several species of Salpa.

In spite of a considerable amount of investigation to determine the presence of nerve cells in the tunicate heart, they were not demonstrated until the publication in 1902 of Hunter's work on Molgula. Hunter succeeded in finding not only bipolar nerve cells but also nerve fibers which run spirally around the heart. The cells are grouped into a ganglion at each end of the heart, and Hunter ('03 a) presents histologic evidence for a connection between them and the central nervous system. Quite recently nerve cells and fibers were also demonstrated by Alexandrowicz ('13) in the heart of Ciona and of A. mentula by the use of methylene blue staining.

The entire wall of the heart is so thin that it precludes the presence of a coronary system. The various elements of the pulsating mechanism must therefore, derive their nourishment directly from the blood which passes through it.

2. Heart beat. The most characteristic feature of the physiology of the heart in tunicates is the periodic reversal of the direction of its beat—a fact first noted by Van Hasselt ('24) in 1821, and since then found to be true for all tunicates. Using the nomenclature suggested by Krukenberg ('80 b), the heart is said to beat in an *advisceral* direction when the contraction wave passes from the branchial end to the visceral end, and in an *abvisceral* direction when the wave passes from the visceral to the branchial end. After beating for a while in one direction the heart

sends out a series of beats in the other direction; the combined pulsations make up a *pulsation series* (Shultze, '01, p. 224).

It is frequently stated, especially in textbooks, that the reversal occurs in such a way that the number of pulsations is the same in each direction (cf. Hunter, '03 b). In Ascidia atra such a state of affairs is not altogether infrequent. An example is given in the following record of the heart beat.

Advisceral	16	20	19	14	
Abvisceral	18	21	18	15	

This, however, is not the normal condition. Most individuals show a marked preponderance in the number of advisceral beats. A truly typical example of this is the heart beat of which the following is the record.

Advisceral	20	44	51	65	
Abvisceral	17	15	31	36	

Here, the number of advisceral beats is nearly twice as great as the number of abvisceral ones. The sum of the beats of the twenty-odd animals which were recorded in my notebook, gives the ratio of advisceral to abvisceral beats as 1.6:1. This is clearly a predominance of the number of advisceral pulsations. The ratio would be even greater were it not that at first I thought the cases of unequal number of beats were abnormal. Consequently, I kept the records of a disproportionately larger number of examples showing equal beats.

The actual number of pulsations which takes place in one direction before reversal occurs, varies with the individual animals. An average of nine measurements on fresh animals gave 23.6 beats in the abvisceral direction and 37.0 beats in the advisceral direction. The abvisceral number varied from 14 to 43 beats and the advisceral from 15 to 83 beats.

Unfortunately A. atra did not prove to be a good laboratory animal. Under the most propitious laboratory conditions, with an abundance of running water, the animals failed to remain normal. For instance, after an individual had been in the laboratory twelve hours or even less, a change was evident in the number of beats in a pulsation cycle. An

average of ten counts on animals which had been in the laboratory for one day or a little longer, gave the number of abvisceral beats as 69.0 and the number of advisceral beats before reversal as 145.9. This is an unmistakable increase over the condition soon after collection.

The only difference of any magnitude between the water in the sea and the water furnished in the laboratory of the Bermuda Biological Station is the complete absence of plankton organisms in the latter. These are precisely what Ascidia uses for food. It would seem, therefore, that the increase in the number of beats as well as the general inability of Ascidia to live in the laboratory is probably due to the immediate starvation to which it is subjected. That this factor may become effective within a relatively short time merely indicates that this species leads a rather "hand-to-mouth" existence.

The literature pertaining to the physiology of the tunicate heart is meagre, but an examination of even these few papers reveals well recorded examples of a preponderance of the advisceral over the abvisceral beats, similar to those which I have presented for A. atra. Roule ('84), for example, showed that the advisceral beats in Ciona intestinalis are three times as numerous as the abvisceral beats. Lahille ('90) found a similar condition in Phallusia mammillata. Here the advisceral pulsations are approximately twice the number of the abvisceral ones.

It might perhaps be supposed that the predominance of advisceral beats is compensated by a difference in the intensity and frequency of the beats in the two directions. Such however is not the case. I could observe no dissimilarity in the intensity of the two beats in any of the individuals of the species. Moreover, although the pulse rate of the advisceral phase is slightly greater than that of the abvisceral, as will be shown presently (see fig. 4), the time occupied by the advisceral phase is still practically twice that required by the abvisceral beats. Similar time relations are true for the two phases of the heart beat in Ciona (Roule, '84).

By the use of drugs and adverse conditions it has been possible to affect the time relations of the two phases of a pulsation series in Salpa (Schultze, '01). In order to avoid the possibility of such effects, I have recorded the pulsation series of animals in their natural locations immediately on collection. There was present the usual preponderance of advisceral beats characteristic of animals which had been taken to the laboratory.

It is, therefore, quite certain that in Ascidia atra, and in at least two other species of ascidians, the quantity of blood which is pumped by the

heart during the advisceral phase of a pulsation series is greater than that pumped during the abvisceral phase of the same series.

3. Pulse rate. The pulse rate is one of the factors which determine the quantity of energy that is expended by an animal in the maintenance of its blood supply. Among various influences, the volume of blood moved in a single pulsation and its respiratory efficiency are perhaps the most important. With regard to the respiratory activity of Ascidia atra, as evidenced by the extent of the water current, it has been shown (Hecht, '16) that the energy expended per unit body weight falls off with an increase in the size of the individual. The volume of blood moved in one pulsation depends on the size of the heart. This has not been determined systematically. The frequency of pulsation and its

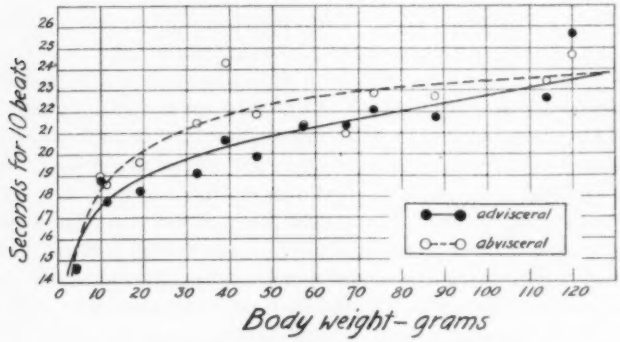

Fig. 4. Relation of pulse rate to weight of animal.

relation to the size of the body have, however, been studied. In figure 4 are plotted the data secured from the hearts of thirteen individuals of varying weight. Altogether, seventy-eight determinations were made, thirty-nine in each phase of pulsation cycle. Three measurements were taken of the time required for ten pulsations to occur in each direction for every animal. The average of the three readings is represented by a circlet in the figure. The smoothed curves in general parallel that obtained for the relation between the extent of the water current and the weight (Hecht, '16). The significance of this is that the pulse rate, which is the reciprocal of the ordinates, varies inversely with the size of the individual. Therefore, in so far as the frequency factor is indicative of the general blood supply, we may conclude that there is relatively less energy devoted to that activity in the larger animals than

in the smaller ones. This conclusion is in harmony with the findings of other investigations of rhythmic activities, such as the respiratory movements of the cloaca of Holothurians (Crozier, '16).

For an understanding of the physiology of the heart beat, however, the most significant feature of the data is the difference in pulse rate between the two phases of a pulsation series. The average of all the readings taken gives the advisceral pulse rate as 29.3 beats per minute and the abvisceral at 28.1 beats per minute. This difference of about 5 per cent in favor of the advisceral phase is not great. But its constancy and regularity, as shown by the two curves in figure 4, serve to emphasize the greater activity of the heart during the advisceral beat. This was already evident in the study of the number of pulsations emitted during the two phases of a pulsation series.

4. Velocity of pulsation wave. The exceptionally long heart of A. atra makes it a convenient object in which to determine the velocity of the pulsation wave. The time taken by a wave to pass over a given distance was determined in three animals of different sizes. The animals were prepared in the usual way, so as to expose only that portion of the heart which lies in a nearly straight line on the right side of the body. The beginning and end of the distance used were sharply marked off by the cut edges of the opaque test. The actual distance was determined at the end of the experiment by placing a piece of fine wire in contact with the heart and bending it into a curve corresponding to that of the organ. The piece of wire was then straightened out and measured. The time interval was determined with a stop-watch.

In all, thirty-eight determinations were made at a room temperature of 27.0°C; nineteen were of the advisceral and nineteen of the abvisceral wave. An equal number of readings in both directions were made on each animal. The figures agreed closely with one another, as shown in the following record:

Experiment I.18. Temperature, 27.0°. Distance of heart exposed 4.4 cm.

Seconds for wave to pass

ADVISCERAL	ABVISCERAL
1.9	2.2
1.9	2.3
1.8	2.2
1.9	2.1
1.9	2.3

An average of the nineteen determinations in each direction gave the velocity of the pulsation wave at 27.0°C. as 2.12 cm. per second in the advisceral direction, and 1.76 cm. per second in the abvisceral direction. This represents a difference of nearly 20 per cent in the velocity of the beat in the two directions, and points again toward the greater activity of the heart while it is beating in the advisceral phase.

5. Significance of unequal phases. All the evidence which has been accumulated in the study of the tunicate heart points unmistakably toward the existence of two centers situated one at each end of the normally acting heart. Moreover, all recent investigators are agreed that the reversal of the direction of the heart beat depends on the alternating dominance in the activity of these two centers.

To show how such a result could be produced on the basis of this assumption, Garrey ('11) has converted the turtle heart into an "Ascidian preparation," whose structure and method of pulsation possess many resemblances to the tunicate heart. By splitting the turtle heart sagittally and keeping the halves united at the apex of the ventricle, a preparation may be made which beats in one direction—from the right heart to the left. This is because of the greater rhythmicity of the right vein, which in the normal heart serves as the pace-maker. If now the rhythmicity of the right vein and the left vein be alternately decreased by vagus stimulation or increased by a local rise in temperature, a periodic reversal in the direction of the pulse wave may be obtained, simulating the normal reversal of the tunicate heart.

In the four aspects of the *normal* heart beat of Ascidia atra which I have just considered, the advisceral phase of a pulsation series shows a decidely greater activity than the abvisceral phase. In terms of the accepted concept of the heart beat this, therefore, means that the advisceral center situated at the branchial end is more powerful than the center situated at the visceral end. Not only does the branchial center initiate pulsations of greater number and velocity, but it maintains this process for a longer time and at a greater rate than does the visceral center.

Aside from the support which this conclusion receives from the data of the normal heart beat, there are other phenomena exhibited by the heart under experimental conditions which also point toward the greater potency of the branchial end. It frequently happens under certain circumstances that another type of beat may be produced in the heart of Ascidia atra. This may be called a central beat because it consists of a series of pulsations originating in the region between the two ends that

normally originate the beats. From this new point of origin two waves proceed simultaneously, one in the advisceral and the other in the abvisceral direction. If such a central beat persists for any length of time, it almost invariably displaces the *abvisceral* phase of the normal pulsation series. For example, the temperature of an animal (I. 23) had been raised from 27° to 30° in ten minutes. At this juncture the central beat appeared. During the half hour in which I observed the animal, not a single abvisceral beat made its appearance. The series of central beats alternated regularly with the advisceral series in a manner resembling the two normal phases of a pulsation cycle.

Not only is it possible to replace the beat with another type of pulsation, but under certain conditions a complete suppression of everything but the advisceral beat may be secured, resulting in a continuous circulation in the advisceral direction.

Such a condition is most readily produced in Ascidia by subjecting the animal to a temperature about 8° higher than that of the room. Another method of accomplishing the same result is to place the individual in a solution that is slightly toxic, such as diluted seawater or $\frac{5}{8}$ M NaCl. I made no attempt to see how long the advisceral circulation could be maintained, but in one experiment (I. 21) it continued for over twenty-one minutes while the animal was kept at a temperature of 35°C.

The difference in potency and viability of the two centers in Ascidia finds its parallel in the differential resistance to drugs of the two centers in the Salpa heart. For example, helleborein reduces the activity of the abvisceral phase to almost nothing, whereas it affects the number of advisceral beats only slightly (Schultze '01, table 27). There are also similar variations in the rhythmicity of different portions of the vertebrate heart. Not only do the different chambers of the vertebrate heart exhibit a difference in the frequency of pulsation, but they also show a differential resistance to various external conditions, notably the hydrogen-ion concentration (Dale and Thacker, '14). Perhaps the example most comparable to the ascidian heart is the variation in activity of two such symmetrical portions of the heart as the two veins of the turtle's heart, the right vein having a greater rhythmicity than the left vein (Garrey, '11).

The greater potency of the branchial end of the heart of A. atra and of the two other species of ascidians previously mentioned is, to my mind, of prime importance for understanding the physiology of the blood supply. The commonly accepted idea is that the heart pumps blood in one direction, and then reverses and pumps an equal quantity of blood—

the same blood, in fact—in the opposite direction. The thesis which I wish to maintain is that, in spite of the periodic reversal of the direction of the beat, there is, in these three species at least, a definite one-way *circulation* of the blood.

It has been pointed out that the quantity of blood which the heart moves in the advisceral direction is about twice the amount which it pumps in the abvisceral direction. The other aspects of the beat, showing the superior potency of the branchial end, merely serve to emphasize this important fact. The blood cells of Ascidia, therefore, *circulate* in the direction of the advisceral beat, except that during the abvisceral phase they are put back in their progress to the extent of one half the distance which they have travelled during the advisceral phase. This corresponds exactly to the kind of progress a man would make who walked along the circumference of a large circle, taking ten steps forward and five steps backward, then ten more forward and five backward and so forth. The resultant of this type of activity would be a forward locomotion leading him around and around the circle in a single direction. Similarly, the greater heart activity during the advisceral phase results in a one-directional circulation in these Ascidians.

An attempt to determine the circulation time of the blood met with no success. Consequently, it is not possible to state how many pulsation series are required for the blood to complete one circulation in the predominating direction.

The existence of this circulation is of interest in an evolutionary connection. The idea that vertebrates arose directly from Ascidians does not receive support from the investigation of the direction of the blood flow. In Ascidia the path of the blood from the heart is away from the gill; in the lower aquatic vertebrates it is toward the gill.

The dominance in the activity of one phase of the pulsation cycle has a distinct bearing on the cause of the reversal of the heart beat. The ideas of many writers on the alternating character of the direction of the blood flow in the tunicates is fairly expressed by the following quotation.

> It has been suggested that the cause of this remarkable reversal may possibly be that the heart being on the ventral vessel, which is wider than the corresponding dorsal trunk, pumps the blood into either the lacunae of the branchial sac or those of the viscera in greater volume than can possibly get out through the smaller branchio-visceral vessel in the same time, the result being that the lacunae in question soon become engorged, and by back pressure cause the stoppage, and then reversal of the heart. The absence of any valves in the heart to regulate the direction of flow obviously facilitates this alternation of the current (Herdman, '04, p. 50).

Somewhat the same idea is at the bottom of the explanation of the change in direction which its discoverer, Van Hasslet ('24), gave. Among others, Todaro ('85), Ritter ('93) and Lahille ('90) have suggested explanations, all based on the same "back pressure" assumption. Lahille ('90) in particular has gone into great detail and has even attempted an experimental proof of this conception.

As Schultze ('01) has already pointed out, this idea would be excellent if there were no *active* contraction of the heart in the reversed direction. On the assumption of the existence of such "back pressure," it would furnish a good explanation if the blood system were a mechanical pumping arrangement run by a physiologically indifferent mainspring. The direction and rhythm would then be dependent on the volume of the containing vessels and on the velocity and quantity of the fluid moving in the apparatus. This, the blood system is not. The heart is an *active* pump. The fact that it may be isolated from the rest of the system—even removed from the body—and still maintain the reversal of its pulsations, shows that the cause of the change in direction of the beat must lie within the heart itself.

Aside, however, from these objections to the back-pressure hypothesis, it seems to me that the assumption on which it is based does not fit the facts which I have summarized with regard to the direction of the circulation. In tunicates there are no blood *vessels* in the ordinary sense of the term. The blood spaces are continuous, and their bore does not vary from pulsation to pulsation as in the blood vessels of the vertebrates. Thus the volume of the blood and the capacity of the blood spaces are constant quantities. Consequently, if any back pressure were to develop due to an inability on the part of certain spaces to transmit the blood supplied by the heart, this back pressure would have to be the same no matter in which direction the heart was beating. Moreover, if a congestion were to result, the quantity of blood required, and therefore the number of pulsations, would have to be equal in both directions before the occurrence of a reversal. Such, however, is not the case, since the quantity of blood and the number of pulsations in the advisceral phase is much more than in the opposite phase.

That the blood is under a definite pressure is undoubted, as I have previously described. But that there is a gradual increase in the blood pressure until congestion sets in, with the resulting reversal to relieve the situation, seems at variance with the findings which these investigations have brought to light. One may as well expect the vertebrate heart to reverse because of the resistance in the capillaries.

6. Nature and origin of contraction wave. In attempting to understand the nature of the heart beat of Ascidia, it is well to keep in mind the distinction between the origin of the contraction wave and its propagation along the heart. The latter phase is the simpler, and a discussion of it will serve to introduce the problem of the basis of the heart activity.

The velocity of the beat along the heart of A. atra is 1.76 cm. per second in the abvisceral, and 2.12 cm. per second in the advisceral direction. The order of magnitude of this velocity is of significance in determining the pathway of the contraction wave.

It is out of the question to compare this velocity with that of the passage of an impulse along well-defined nerve trunks like those found in vertebrates. Such structures certainly do not exist in the heart. The only demonstration of nerve tissue at all in the heart of an Ascidian is the work of Hunter ('02). Alexandrowicz ('13) merely states that he saw nerve cells but gives no figures to show their distribution.

On the tentative assumption that there is a type of loose nerve-net in the heart, it is clarifying to examine the velocities that have been obtained for such a type of conduction. A well-defined case of nerve-net conduction is the passage of the wave in a ring of the disc of the medusa Cassiopea. At 25°C. the velocity of this conduction varies from 27.6 to 49.9 cm. per second (Harvey, '11). The slowest conduction obtained in nerve tissue by Jenkins and Carlson ('04) was in the ventral chain of Cerebratulus. This is a nerve-net, and the velocity of transmission along the chain varied from 5 to 9 cm. per second. The ventral chain of other worms showed a much greater velocity of transmission: Nereis gave 89 cm. per second and Bispira 694 cm. per second. In short, even the slowest velocities of nerve-net conduction are at least three times as great as that found in the heart of Ascidia.

The only case in which the nervous nature of the transmission of the contraction wave has been unmistakably demonstrated is the heart of Limulus (Carlson, '04). In this animal the velocity of propagation is so great that the heart, half a foot long, seems to contract as a single unit. Only under special circumstances is it possible to show that the beat arises in the posterior third of the heart and travels forward along a nerve-net which lies on the outside of the heart (Carlson '04, p. 70). The magnitude of the velocity of the heart beat of Ascidia would therefore seem to prove that we are dealing with a slow, muscular transmission, comparable to the wave of muscular contraction which causes the locomotion of the earthworm (Friedländer, '88).

The nature of the activity which underlies the *origin* of the beat is complicated by several factors. As already stated, the immediate cause of the curious behavior of the ascidian heart is most probably the alteration in the potency of its two ends (J. Loeb, '02, p. 29). The origin of a single beat is therefore due to the activity of the center situated at one of these ends. This idea was first expressed by J. Loeb ('02) on the basis of Lingle's work on Molgula (Bancroft and Esterly, '03). J. Loeb describes how the source of automatic activity is confined to a small region at each end of the heart. The discovery of a mass of nerve cells in these spots in the Molgula heart (Hunter, '02) seemed to prove that the initiation of the heart rhythm is due to the activity of the nerve cells. This neurogenic idea was emphasized by Lingle's (J. Loeb, '02) inability to cause the central portion of the heart to beat in seawater, and the comparative sparseness of nerve cells found by Hunter ('02) in this region.

Further investigation, however, showed that some of this work was in error. For example, both Schultze ('01) and Bancroft and Esterly ('03) found no difficulty in securing an automatic rhythmicity of the isolated central part of the heart in ordinary seawater. Later, Hunter found the same to be true for the species with which Lingle had first worked (Hunter, '03b).

Similarly, the isolated central portion of the heart of Ascidia atra beats in seawater. Moreover, not only can the isolated central part of the heart initiate automatic activity, but this portion may produce beats even when the heart is in its normal position in the intact animal. Careful observation has convinced me that this central beat may originate in practically any place between the two ends, although it frequently occurs at the node. This may happen not merely in hearts which have been subject to slightly toxic conditions but is frequently to be observed in freshly captured animals.

In terms of a neurogenic conception of the heart beat, these facts have no significance. If, however, we adopt the myogenic conception, viz., that all the heart muscle cells are capable of an automatic rhythm, the situation at once becomes clear. The ability of any portion to initiate pulsations for the entire heart depends upon its rate of pulsation and upon the refractive properties of heart muscle. In the normal Ascidia heart the two ends alternately act as pace-makers, due to the alternating superiority of their pulse rates. The balance in their favor over the central portion of the heart is, however, so delicate that it requires only a slight change in metabolic activity of the heart to upset it. The

branchial end is normally the most potent part of the heart, as is evidenced by the much greater duration and rate of its activity. This probably signifies so large a balance in its favor that, unlike the visceral end, it is never replaced by a central beat in the regular pulsation cycle.

The fact that external stimulation may change the rhythm and intensity of the heart beat of Ascidia and of Salpa (Nicolai, '08), is no argument for the nervous nature of the origin of the beat. Such changes are entirely comparable to the effect of vagus stimulation of the vertebrate heart, the beat of which has been shown by the experiments of Burrows ('12) and of Hooker ('11) to be undoubtedly of muscular origin.

It is possible, therefore, to summarize the discussion in this section by the following conclusion. The heart beat of Ascidia atra originates within the muscle cells, and the conduction of the pulsation wave is accomplished by the passage of the impulse across a continuous muscular pathway.

7. Temperature and heart activity. The general effect of a change in temperature on the cardiac activity of Ascidia atra serves as a demonstration of the balance maintained by the different parts of the heart. The precision of this balance undoubtedly varies among individuals and it is therefore not surprising that the degree of disturbance produced by a change of temperature should be different in individual cases. In some animals, for example, a change in temperature may produce no other than an accelerating or retarding effect depending on the direction of the change; in others additional phenomena may make their appearance.

Some reference has already been made to a central beat which appears under these conditions. If this beat is only occasional, it may occur between the usual phases of a pulsation cycle. If it persists, it will often supplant the abvisceral beat completely. These results can be obtained with a decrease in temperature as well as with an increase, although the latter is a more efficient means.

An effect on the color of the blood, not directly connected with heart activity, is produced by a change of temperature. The clotting of the blood of Ascidia within the intact blood system has already been described. One way of producing this agglutination is to raise the temperature of the animal several degrees. The blood becomes green and to all appearances behaves as if it had been clotted by a vigorous external stimulation of the animal. The color change occurs at a point (32.0°) well below the death temperature of the animal, and consequently is not due to a heat coagulation of either the plasma or the corpuscles.

This is further assured by the reassumption of the usual transparency with continued warming.

The temperature limits of heart activity show a marked individual variation which in all probability depends upon the rate of the temperature change and the duration of the exposure. For example, in one experiment the cooling was performed at the rate of about one degree for every ten minutes. The lowest temperature at which the heart maintained its beat was 13.0°C. In a similar experiment the cooling was accomplished twice as rapidly, and then the lowest temperature at which the heart was active was 17.1°C. These experiments were performed on two occasions only, and are consequently merely suggestive of the imperative consideration to be given to the time factor in determinations of temperature limits. As has been pointed out by Crozier ('16, p. 329), attention to such sources of error is, however, all too uncommon in biologic investigations.

Bearing this in mind, we may say that the range of temperature over which the heart of Ascidia is active is about 20°. The lower limit varies around 15°; the upper limit is near 36°C.

The effect of the temperature is evident in a change in the pulsation rate and in the velocity of the contraction wave. Both of these aspects of the cardiac activity admit of such accurate measurement that it seemed desirable to investigate quantitatively their relation to the temperature.

The method adopted was simple, uniform and avoided certain sources of error. The animal was in a beaker of seawater, which in turn was suspended in a larger dish of water. The large dish received the brunt of the warming and cooling, the temperature being equalized in the system by constant stirring. At selected temperatures measurements were made in each direction of the beat: five of the velocity or three of the pulsation rate, depending on the experiment. The actual procedure was to make determinations at room temperature, then to cool the animal about three degrees in ten minutes, and to maintain it at this temperature for ten minutes. During the last two minutes of this period readings were again made, after which the cooling continued until the next selected temperature was reached. This was again maintained for ten minutes, and so on, the process being repeated until the lowest temperature was recorded. Then the animal was allowed to return slowly to room temperature. After a few hours the warming was begun, the experiment proceeding along the same lines as before.

The relation of the temperature to the time required for ten beats is

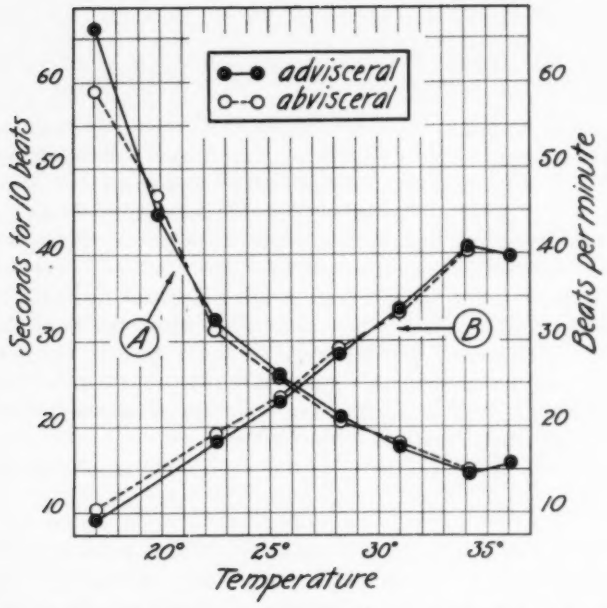

Fig. 5. Relation of pulse rate to temperature. *A*, number of seconds required for ten beats; *B*, number of beats per minute.

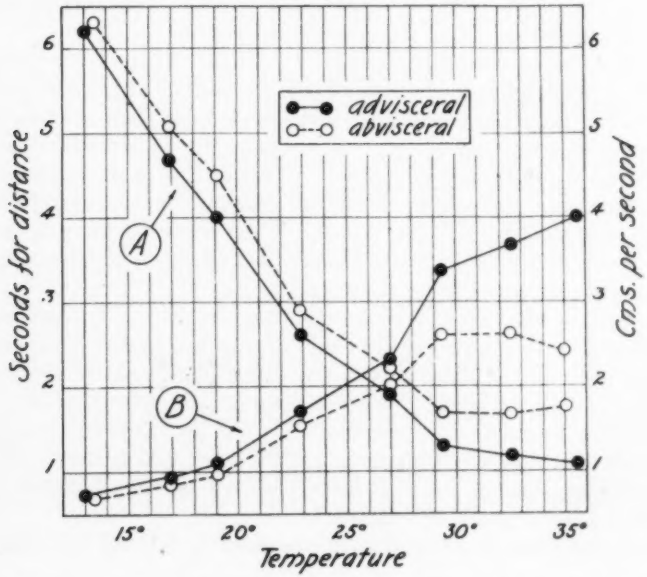

Fig. 6. Relation between temperature and velocity of contraction wave of heart of Ascidia. *A*, number of seconds required for a wave to pass across a given distance; *B*, velocity of the wave in centimeters per second.

given for one animal in curve A of figure 5. It is entirely typical of the other experiments. Curve A of figure 6 expresses similarly the average results of an experiment on the relation of temperature to the velocity of pulsation. This may best be shown as the time required for a contraction wave to pass over a given distance along the heart. The two curves resemble the numerous figures that have already been drawn to show the relation of temperature to various life processes (Kanitz, '15).

Although there has been much controversy about the exact shape of such curves in general, the matter is far from settled. Snyder ('13), among others, has consistently supported the idea that they are exponential in character, whereas Krogh ('14), for example, has interpreted similar results as linear functions of the temperature.

A minor source of confusion in this connection is the difference in the methods of recording data. It will be remembered that $y = \frac{1}{x}$ represents the equation for an hyperbola referred to its asymptotes as axes, and that $y = x$ is the equation of a straight line. Consequently, much depends on whether the experimental value itself or its reciprocal is used in plotting the results. Figure 5 represents just such a situation. Curves A and B have the same abscissas, whereas the ordinates of one are the reciprocals of those of the other. In the one case the curves resemble an hyperbola; in the other, a straight line.

Only a few of the discrepancies (cf. Krogh, '14, p. 170), however, are to be explained on the basis of this confusion. In Ascidia, for example, two phases of the activity of the same organ show the two types of results illustrative of the effect of temperature. Curves B of figure 5 show that the pulse rate is clearly a linear function of the temperature. Its temperature coefficient is therefore constant for the complete range of its normal activity. The velocity of the pulsation wave, however, is an exponential function of the temperature (B, fig. 6), and its temperature coefficient varies from 2.1 to 2.9.

In spite of the heated controversy (Snyder, '13, p. 77, footnote), it is difficult to see wherein either result is possessed of any great significance. The earlier investigators were much impressed by the resemblance between the temperature coefficient of protoplasmic activity and of chemical reactions. On this similarity was based the conclusion, for example, that the underlying process of the heart beat is a single chemical reaction (Harvey, '11). It must, however, be clear at present that this is much too simple an explanation.

Any protoplasmic movement is composed of several chemical and

physical reactions, which may differ in direction, velocity and intensity (cf. especially, Pütter, '14). A few of the more obvious physiologic factors which are concerned in the heart beat of Ascidia atra are: the origin of the internal stimulus, its propagation through the cell, the refractory period of the muscle and the degree of its irritability. Each of these is undoubtedly conditioned by several reactions which result in an interplay of several kinds of energy. A change of temperature has an individual effect on these numberless reactions, and it is their resultant which is expressed by the relation of the temperature to the frequency of the pulsation.

The remarkable thing, to my mind, is not whether the relation between temperature and pulse rate is linear or exponential, but that there exists any definite relation at all. The real significance of temperature effects will not become clear from a superficial comparison with simple chemical reactions. Their interpretation will come only after an analysis of the principal reactions concerned in protoplasmic activity, much as the understanding of the timbre of a musical sound is the result of its resolution into the tones and overtones combined in its production.

IV. SUMMARY

1. The blood of Ascidia atra is colorless and transparent. It flows through the body under an appreciable pressure.

2. The blood plasma is isotonic with seawater.

3. The blood has an acid reaction; the acidity is resident in the green corpuscles and not in the plasma.

4. There are at least two kinds of cells in the blood: pigmented and unpigmented. Of the pigmented cells, the green are distributed all over the body; the orange are localized in the branchial sac; and the blue are found in the regions of the viscera, etc. Some of the unpigmented cells are ameboid; others are not.

5. In the green cells, the pigment is a compound containing vanadium, probably in a stage of oxidation corresponding to V_2O_3. It is not a respiratory pigment but is most likely of value as a catalytic agent.

6. The coagulation of the blood depends on the agglutination of its cells. Clotting often occurs within the intact blood system as a result of vigorous external stimulation.

7. The heart of Ascidia atra is long and has a node which divides it unequally. The presence of the node may be demonstrated physiologically as well as anatomically.

8. A pericardial body is present.

9. In most of the animals a pulsation series shows about twice as many advisceral beats as abvisceral beats.

10. The pulse rate decreases as the size of the animal increases. It is greater in the advisceral phase than in the abvisceral phase.

11. The velocity of the contraction wave is greater in the advisceral direction than in the abvisceral direction.

12. The greater activity of the heart during the abvisceral phase of a pulsation cycle indicates that, in spite of the periodic reversal of direction, there is a resultant *circulation* of the blood in the advisceral direction.

13. These facts are incompatible with the "back pressure" explanation of the periodic reversal of the heart beat. They are, however, in harmony with the idea that the reversal is due to the alternating dominance as pacemakers of the two ends of the heart.

14. The presence of a central beat, the suppression of the abvisceral beat and the magnitude of the velocity of the pulsation wave indicate that the heart beat is myogenic, and that the contraction wave passes along the muscular elements across the heart.

15. The pulsation rate is a linear function of the temperature, whereas the velocity of the pulsation wave is an exponential function of the temperature. Neither relation is shown to be of real significance in view of the complexity of reactions involved in the heart beat.

BIBLIOGRAPHY

ALEXANDROWICZ, J. S. '13 Zeitschr. allg. Physiol., xiv, 358.

ALSBERG, C. L., AND M. W. CLARK. '14 Journ. Biol. Chem., xix, 503.

BANCROFT, F. W., AND C. O. ESTERLY. '03 Univ. Calif. Publ. Zoöl., i, 105.

BETHE, A. '14 Bull. Inst. océan. Monaco. no. 284.

BURROWS, M. T. '12 Sci., N. S., xxxvi, 90.

CANNON, W. B. '15 Bodily changes in pain, hunger, fear and rage. New York.

CARLSON, A. J. '04 This Journal, xii, 67.

CROZIER, W. J. '16 Journ. Exper. Zoöl., xx, 297.

CUÉNOT, L. '91 Arch. Zool. Expér., Sér. 2, ix, 13.

DALE, D., AND C. R. A. THACKER. '14 Journ. Physiol., xlvii, 493.

DREW, G. H. '10 Quart. Jour. Micr. Sci., liv, 605.

FERNANDEZ, M. '06 Jena. Zeitschr., xli, 1.

FRIEDLÄNDER, B. '88 Biol. Centralbl., viii, 363.

FÜRTH, O. VON '03 Vergleichende chemische Physiologie der niederen Tiere. Jena.

GARREY, W. E. '11 This Journal, xxviii. 330.

GAVER, F., VAN AND P. STEPHEN. '07 C. R. Soc. Biol., lxii, 554.
GRIFFITHS, A. B. '92 C. R. Acad. Sci., cxv, 738.
HARLESS, E. '47 Arch. Anat. Physiol., 148.
HARVEY, E. N. '11 Carnegie Instn. Publ., no. 132, 27.
HASSELT, J. C. VAN. '24 Ann. Sci. Nat., Zool., iii, 78.
HECHT, S. '16 Journ. Exper. Zoöl., xx, 429.
'18 a Journ. Exper. Zoöl., xxv, 229.
'18 b Journ. Exper. Zoöl., xxv, 261.
HELLER, C. '75 Denk. Akad. Wiss. Wien. Math.-Nat. Classe, xxxiv, Abt. 2, 1.
HENZE, M. '11 Zeitschr. physiol. Chem., lxxii, 494.
'12 Zeitschr. physiol. Chem., lxxix, 215.
'13 Zeitschr. physiol. Chem., lxxxvi, 340.
HERDMAN, W. A. '04 Cambridge Nat. Hist., vii, 33.
HÖBER, R. '14 Physikalische Chemie der Zelle und der Gewebe, Leipzig und Berlin.
HOOKER, D. '11 Journ. Exper. Zoöl., xi, 159.
HOWELL, W. H. '12 Textbook of physiology, Philadelphia.
HUNTER, G. W. '02 Anat. Anz., xxi, 241.
'03 a Sci. N. S., xvii, 251.
'03 b This Journal, x, 1.
JENKINS, O. P., AND A. J. CARLSON. '04 Journ. Comp. Neurol., xiii, 259.
KANITZ, A. '15 Temperatur und Lebensvorgänge, Berlin.
KROGH, A. '14 Zeitschr. allg. Physiol., xvi, 162.
KRUKENBERG, C. F. W. '80 a Vergleichend-Physiol. Studien, I. Reihe, Abt. iii, 66.
'80 b Vergl.-Physiol. Studien, I. Reihe, Abt. iii, 151.
LAHILLE, F. '90 Contributions a l'étude anatomique et taxonomique des Tuniciers. Thèse, Paris.
LOEB, J. '02 Comparative physiology of the brain and comparative psychology, New York.
LOEB, L. '10 Biochem. Zeitschr., xxiv, 478.
NICOLAI, G. F. '08 Arch. Anat. Physiol., Physiol. Abt., Suppl. Bd., Jahrg. 1908, 87.
PÜTTER, A. '07 Zeitschr. allg. Physiol., vii, 283.
'14 Zeitschr. allg. Physiol., xvi, 574.
RITTER, W. E. '93 Proc. Calif. Acad. Sci., Ser. 2, iv, 37.
ROULE, L. '84 Ann. Musée Hist. Nat. Marseille, Zool., ii, 1.
ROUX, W. '96 Arch. Entw.-Mech., iii, 381.
SCHULTZE, L. S. '01 Jena. Zeitschr., xxxv, 221.
SEELIGER, O. '93–11 Tunicata. Bronn's Klassen und Ordnungen des Tier-Reichs, iii, (suppl.), Abt. 1, 1.
SNYDER, C. D. '13 Zeitschr. allg. Physiol., xv, 72.
TODARO, F. '85 Atti R. Accad. Lincei, ser. 4, Mem. i, 641.
VERNON, H. M. '95 Journ. Physiol., xix, 18.
WILSON, H. V. '11 Journ. Exper. Zoöl., xi, 281.
WINTERSTEIN, H. '09 Biochem. Zeitschr., xix, 384.
WITZ, G. '76 C. R. Acad. Sci., lxxxiii, 348.

EVIDENCE FOR THE ENZYMATIC BASIS OF HEART-BEAT[1]

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Received for publication December 6, 1917

Among the many investigators who studied the effect of temperature on the heart-beat, Cyon (1) did the most conclusive of early work. He studied the effect of changes of temperature on the number, duration and strength of the beats of the excised frog heart. He found that the rate increased regularly with the temperature until the number of beats reached a maximum. After reaching this point the rate became irregular and slowed rapidly so that the heart came to rest only a few degrees above the maximum, between 30° and 40°. These observations were extended and confirmed for the mammalian heart by Newell Martin (2) and by Langendorff (3). G. N. Stewart (4) found that extreme high temperatures cause first an increase in rate to a maximum and later a decrease followed by standstill in the hearts of the frog, toad and turtle. The same was found to be true of the terrapin heart by E. G. Martin (5).

The problem took on a new aspect with the application of the law of van't Hoff and Arrhenius to biological reactions. This law applies to chemical reactions and states that for every increase of ten degrees in temperature the rate of reaction is doubled or trebled. A great number of investigators have established the fact that metabolic processes are influenced by heat in the same direction and degree as chemical reactions, and they have shown that the law holds for medium temperatures (10° to 27°). The conclusion arrived at is that life activities are at bottom chemical.

The question at issue is, Does the law hold for higher temperatures, and in case of deviation, what are the modifying factors? The heart-beat of crustacea (6), of cold-blooded (7) and warm-blooded vertebrates (8) has been shown to obey this law within the medium range. If the law holds for higher temperatures, the heart ought to beat with

[1] Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Rutgers College.

increasing velocity as the temperature is raised until it abruptly stops at the critical coagulation point.

Loeb (9) long ago suggested that the heart owes its rhythmicity to the "constant fermentative production of certain compounds in the automatically active cell." If this idea is correct, the curve of the rate of heart-beat plotted against temperature should be a typical enzyme curve and the temperature coefficient of the rate of heart-beat should show the same variations which that of enzyme reactions do, i.e., become smaller with increasing temperature. The rate of reaction should increase more rapidly at low temperature than at medium temperature; should then obey the law of van't Hoff and Arrhenius for the middle range; should reach a maximum and finally fall off to zero with further rise in temperature. In the last few degrees in which any reaction is detected, there should be a negative temperature coefficient. It is known that the rule of van't Hoff holds for enzymes generally between 15° and 35°. Above 35° and below 45° the increase in velocity of reaction is greater than the law predicts, while above 45° there is a rapid fall possibly due to the destruction of the enzyme at the high temperature (10). The position of the maximum and the killing temperature varies a great deal with different methods and different ferments but the characteristic effects of temperature on the rate of the reaction is the same in all cases.

THE EFFECT OF TEMPERATURE ON THE RATE

Dr. Loeb suggested to me the possibility of determining whether the law of van't Hoff and Arrhenius holds for the heart-beat at high temperatures or whether the course of the reaction is like that of an enzyme. Accordingly, in the summer of 1917, I carried out a series of experiments on Fundulus embryo-hearts.

Fundulus heteroclitus embryos were used from the time the heart had begun to beat regularly until it was obscured by the developing pigment, i.e., when the embryos were from seven to fourteen days old. The embryo was placed in a small Petri dish holding 10 cc. of seawater, the temperature of which was varied by means of a surrounding water-bath. The bulb of a thermometer was placed in the small Petri dish as near as possible to the embryo. The time for twenty beats of the auricle was taken with a stop-watch after the mercury had registered a convenient temperature continuously for about two minutes. The embryo was kept under observation during the entire course of the experiment, the temperature being gradually but steadily raised from

room temperature or a little below, to the point at which the heart ceased beating altogether. From 32° to 46° the readings were taken as rapidly as possible.

The results of these experiments are illustrated by the experiments and their corresponding graphs given below. The rate, calculated as $\frac{100}{\text{Time for 20 beats,}}$ is plotted against temperature (see tables and figures 1 and 2).

The rate increases quite regularly with the temperature from 10° to 40°. Above 40° the rate suddenly increases more rapidly than before, and above 41° to 43°, the curve falls to zero. The exact position of the maximum depends upon the length of time exposed to the high temperature, and upon the length of time taken to raise the temperature to a high level. This time factor will be discussed later. The shorter the time, the higher the maximum. As nearly as was possible the length of time taken up by each experiment was kept at about fifteen minutes in all.

THE TIME FACTOR

Some experiments were made in which the temperature of the water-bath was lowered from 46° to room temperature, instead of raised from a lower to a higher temperature. One of these experiments, number 9, is quoted below (see table 3).

It is evident that in this experiment the influence of length of exposure is shown. At 45° and 46° the rate steadily decreases. When the temperature is lowered, the rate increases to a maximum and the temperature coefficient becomes positive. The factor of length of exposure must be important at temperatures near the death point of the organism; but it is negligible at medium or normal temperatures since the animal spends a lifetime at such temperatures. Using the method described by Loeb and Ewald (11) in their experiments on the effect of temperature on the heart-beat of Fundulus embryos, I determined the temperature at which the rate of heart-beat was greatest after from fifteen to twenty minutes in a thermostat. The maximum rate was found at 32° or 33°. The maximum is much higher in the above results because the time of exposure has been greatly lessened. At temperatures higher than the optimum the time factor becomes greater with each degree. If the injury due to length of exposure were zero, as at temperatures below 32°, the rate would continue to rise with the temperature until the coagulation point of the tissues is reached.

As a matter of fact, injury by exposure intervenes, just as in an enzyme reaction, and slows the rate before an observation can be made, since the time factor cannot be completely eliminated. The slowing of the

TABLE 1

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
10.0	33.2	3.0
12.5	27.0	3.7
16.0	17.6	5.6
18.0	14.8	6.7
20.0	12.0	8.3
24.0	9.0	11.1
27.0	7.5	13.3
29.0	6.6	15.1
32.0	5.6	17.8
36.0	5.2	19.2
39.0	4.0	25.0
42.0	3.6	27.7
43.0	3.0	33.3
44.0	2.8	35.7
45.0	3.2	31.2
46.0	4.0	25.0
47.0	4.2 stopped	23.8

TABLE 2

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
11	27.4	3.6
15	15.4	6.5
20	10.0	10.0
24	7.5	13.3
28	6.4	15.6
33	5.0	20.0
35	4.8	20.8
38	4.2	23.8
40	3.6	27.7
42	3.0	33.3
43	2.6	38.4
44	3.2	31.2
45	4.0	25.0
46	stopped	

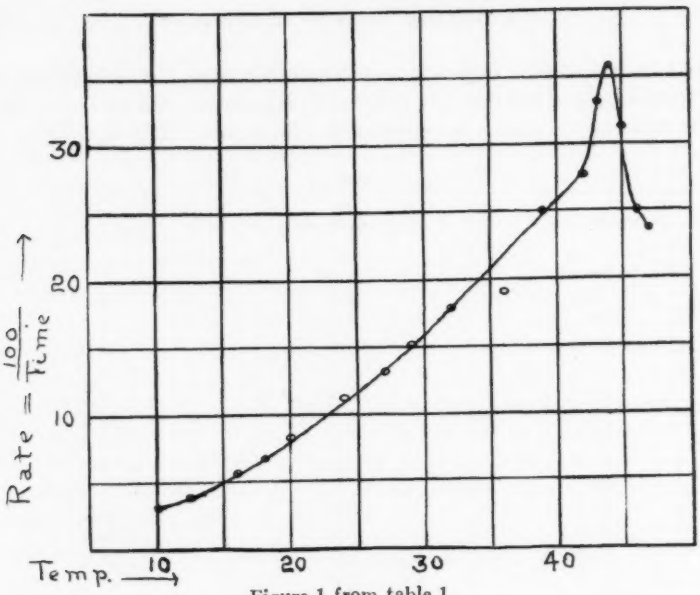

Figure 1 from table 1

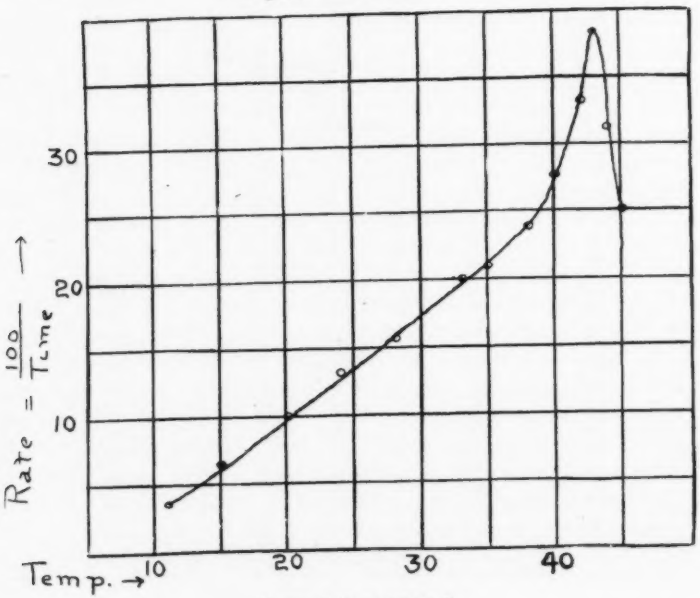

Figure 2 from table 2

rate comes before the coagulation process has become irreversible, or possibly before any coagulation has occurred. The heat may partly destroy the enzyme before it has killed the cells, in which case complete recovery of the heart should occur when the temperature is lowered even if the heart has altogether ceased to beat. This in fact is the case and can be observed with any embryo.

If the embryo is kept at a constant high temperature and observed continuously, the rate of heart-beat is seen to increase at first, then to decrease and finally to fall to zero. This is shown by the following experiment (see table 4).

TABLE 3

Experiment 9

TEMPERATURE	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees	*seconds*	
46 (1)	6.0	16.6
(2)	7.5	13.3
45	8.0	12.5
43	4.0	25.0
42	3.8	26.3
41	3.2	31.2
40	4.0	25.0
39	4.0	25.0
36	4.4	22.7
34	5.0	20.0
32	5.0	20.0
30	5.2	19.2
28	5.8	17.2
26	8.0	12.5
25	8.8	11.3
23	9.8	10.2
19	15.4	6.5

THE TEMPERATURE COEFFICIENT

C. G. Rogers (12) has shown that the temperature coefficient of the rate of heart-beat of the Fundulus embryo is of the order of magnitude of a chemical reaction. Loeb and Chamberlain (13) pointed out that the temperature coefficient of the rate of heart-beat agrees with the supposition that the heart-beat is determined by the velocity of an enzyme reaction. The temperature coefficients of various enzyme reactions have been determined for the entire temperature range of

their action (14). The values of Q_{10} in all these cases are higher for temperatures near zero and decrease as the temperature is raised.

The following table (table 5) gives in terms of beats per minute the average rates of heart-beat from a large number of observations. Previous to making the observations the embryos were kept in a thermostat at the desired temperature for from fifteen to twenty minutes.

TABLE 4

TEMPERATURE	MINUTES AFTER BEGINNING EXPERIMENT	TIME FOR 20 BEATS	RATE = $\frac{100}{\text{TIME}}$
degrees		*seconds*	
44		3.8	26.3
45	1	5.0	20.0
45		6.6	15.1
45	2	7.0	14.2
45		7.2	13.8
45		7.5	13.3
45	3	7.7	13.0
45	4	7.8	12.8
45	5	8.0	12.5
	6	stopped	

TABLE 5

TEMPERATURE	BEATS PER MINUTE	Q_{10}
2.5–12.5	53.2/ 7.0	7.6
5.0–15.0	69.0/ 19.0	3.6
7.5–17.5	86.3/ 29.7	2.9
10.0–20.0	100.0/ 41.1	2.4
12.5–22.5	122.5/ 53.2	2.3
15.0–25.0	139.0/ 69.0	2.0
17.5–27.5	180.9/ 86.3	2.0
20.0–30.0	200.0/100.0	2.0
22.5–32.5	223.5/122.5	1.9
25.0–35.0	200.0/139.0	1.4

The temperature coefficients are calculated for 10 degree intervals. The resemblance of these values to the temperature coefficients of enzyme action is obvious. In this connection it is interesting that E. N. Harvey (15) determined the effect of temperature on the pulsations of the medusa Cassiopea xamachana, and found that the temperature coefficient resembles closely that of an enzyme action.

HEART-BLOCK

If the temperature of the heart is gradually raised, a point is reached at which the normal rhythmical sequence of the parts no longer occurs. G. N. Stewart (16) observed heart-block as the result of heat in the case of the frog. With Fundulus embryos a block develops so that regularly not all of the beats of the auricle are followed by ventricular contractions. If the heart is not heated too suddenly, the block develops in well-defined steps. The ratio of auricular to ventricular beats may be successively, 6/5, 5/4, 4/3, 3/2, 2/1, 3/1, etc., until the ventricle stops beating altogether. If the temperature is then slowly lowered, the reverse changes in the ratios take place until the normal sequence is resumed. The temperature at which the block appears is two or three degrees above that at which the block disappears upon lowering the temperature. The exact point at which the block appears depends upon the former treatment of the embryo. Embryos which had been kept in a refrigerator with a temperature of from 8° to 10° for several days, when gradually heated developed heart-block at from 28° to 29° in every instance. Embryos which had been kept at room temperature developed block at from 34° to 35°, while embryos which had been kept at 34° to 35° for an hour and had recovered from the block at that temperature, had normally beating hearts up to 41° or 42°. If the embryo is kept for some few minutes at the temperature at which the block appears, the heart recovers its normal beat, and in order to produce block again, the temperature must be further raised. I never observed a recovery above 39°, however. One embryo was kept at 39° without affecting the block, for an hour and a quarter, when the experiment was discontinued. After a half hour, during which the temperature had fallen to that of the room, the heart had completely recovered its normal beat.

The ventricle is unable to beat at temperatures above 42°. The auricle stops beating at from 44° to 46°, according to the length of time exposed, and the sinus stops immediately after the auricle. It was difficult to determine this point exactly since the beats of the sinus, besides being very rapid at high temperatures, become weak and almost indistinguishable. In several experiments the sinus was seen to beat two or three times immediately after the auricle had ceased beating, and then to come to rest, relax and fill with blood from the auricle and ventricle. In every case after standstill of the heart, the sinus was full of blood so that the spot was visible to the naked eye.

CONCLUSIONS

1. The changes in the rate of the heart-beat of the Fundulus embryo at high temperatures are such as would be expected in case the rhythmical contractions of the heart depend upon the velocity of an enzyme reaction.

2. With high temperatures the length of time exposed is an important factor. The longer the time exposed, the lower the temperature necessary to bring about standstill of the heart, indicating a temperature coefficient of the destruction of the enzyme.

3. The values of Q_{10} are shown to be higher at temperatures near zero and to decrease, as is the case with enzymes, when the temperature is raised.

4. Auriculo-ventricular block was observed as a result of high temperature. The ventricle is first affected by the high temperature, and finally the auricle and sinus.

I wish to thank Professor A. R. Moore for constant encouragement and many helpful suggestions in carrying out this work.

BIBLIOGRAPHY

(1) Cyon: Gesammelte Physiologische Arbeiten, Berlin, 1888.
(2) Martin: Phil. Trans. Roy. Soc., London, 1883, clxxiv, 663.
(3) Langendorff: Pflüger's Arch. f. Physiol., 1897, lxvi, 355.
(4) Stewart: Journ. Physiol., 1892, xiii, 22.
(5) Martin: This Journal, 1904, xi, 370.
(6) Robertson: Biol. Bull., 1906, x, 242.
Snyder: This Journal, 1906, xvii, 350.
(7) Snyder: Univ. Cal. Publ. Physiol., 1905, ii, 125.
Rogers: This Journal, 1911, xxviii, 81.
(8) Snyder: Zeitschr. allg. Physiol., 1913, xv, 72.
(9) Loeb: Comparative physiology of the brain, New York, 1900, 23.
(10) Taylor: Univ. Cal. Publ. Path. 1907, i, 112.
Euler: General chemistry of the enzymes, (transl. by Pope), New York, 1912, v.
(11) Loeb and Ewald: Biochem. Zeitschr., 1913, lviii, 177.
(12) Rogers: Loc. cit.
(13) Loeb and Chamberlain: Journ. Exper. Zoöl., 1915, xix, 559.
(14) Slator: Journ. Chem. Soc., 1906, lxxxix, 128.
Van Slyke and Cullen: Journ. Biol. Chem., 1914, xix, 174.
Harris and Creighton: Journ. Biol. Chem., 1915, xx, 179.
(15) Harvey: Carnegie Inst. of Wash. Publ. no. 132, Papers from the Tortugas Laboratory, III, 1911, 29.
(16) Stewart: Loc. cit.

VASODILATOR REACTIONS. I

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Received for publication December 7, 1917

The experiments described in the following resulted from an endeavor to discover the cause of the marked fall of blood pressure following the administration of certain drugs; they have led to the conclusion that there is present in mammals (and perhaps other animals) a vasodilator mechanism not recognized, or only vaguely recognized, capable of responding with more intense reactions than any of the mechanisms generally recognized and one which may be more perfectly controlled than the latter.

The present work began with the discovery by Taveau and myself in 1906 of the intense blood pressure lowering action of acetyl-cholin.[1] In our first publication (1) on this subject I said,

> I think it safe to state that, as regards its effect upon the circulation it (acetyl-cholin) is the most powerful substance known. It is one hundred thousand times more active than cholin, and hundreds of times more active than nitroglycerin; it is a hundred times more active in causing a fall of blood pressure than is adrenalin in causing a rise.

[1] In our paper in 1906 Taveau and I described a physiological test for cholin, based upon its conversion into the acetyl compound, by means of which 0.0001 mgm. or less of cholin could be detected. This method was later elaborated (2) using the isolated frog heart as a test object so that 0.00001 mgm., and probably less, cholin could be detected; a number of applications of the method were described. Ten years after our first description of this method Guggenheim and Loeffler (3) without knowing of my work (as stated in a letter from Doctor Loeffler) published a similar method making use of the guinea pig intestine as a test object. This method seems (I have not seen the original paper) to be far less sensitive than mine; but although these authors do not seem to have taken the precautions I did to avoid splitting off cholin from the lecithin of the serum the figures given for the cholin content of the blood serum (from 0.2 to 2.0 mgm. per 100 cc.) are similar to those I found. Their observations upon the rapid disappearance of cholin from the blood and upon the amount present in the urine also agree with mine. Führer (4) is quoted as having emphasized the superiority of the frog heart as a test object.

These results were fully substantiated in subsequent work and were confirmed eight years later by Dale (13). Figure 1 shows the effect of 0.000,000,002,4 mgm. of acetyl-cholin per K upon the blood pressure when injected intravenously into a cat. The response was, I believe, more active in this experiment than in any other; the stated dose caused the same fall of pressure repeatedly. Injections of equal amounts of the 0.9 per cent sodium chloride solution used in making the acetyl-cholin solution, had no effect. In order to avoid the possibility of an error in the dilution a fresh series of dilutions was made; the results were the same.

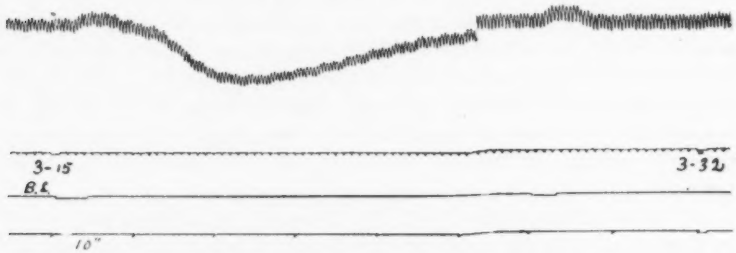

Fig. 1. Experiment 359. Cat, 4.16 K; paraldehyde; vagi cut. Blood pressure from left femoral artery; injections into right saphenous vein. *3–15*, 1 cc. of acetyl-cholin 1:100,000,000,000. *3–32*, 1 cc. 0.9 per cent sodium chloride solution.

Taveau and I also described in 1906 a chemical test for cholin based upon its conversion into the benzoyl compound and the precipitation of the latter with platinum chloride; this test seemed to have advantages over the tests then in use (cf. 5).

My work on the cholin esters was suggested by some work I had done previously which had resulted in the isolation of cholin from adrenal extracts and the identification of it as the chief substance causing the fall of blood pressure of such extracts after the removal of the epinephrin (6)—an observation frequently ascribed to Lohmann, but the publication of my work antedated that of Lohmann by seven years. At that time no one, apparently, except Marino-Zucco (who had reported finding "neurin," evidently cholin, in the adrenal glands) had isolated cholin from any organ extract and the thought at once occurred to me that possibly this substance might represent an internal secretion of the cortex in the same way that epinephrin was supposed to be an internal secretion of the medulla. Further work (7) (1) suggested that there may be present in the adrenal glands compounds of cholin much more active than cholin itself and this led me to prepare and study pharmacologically a few cholin esters already known and later, in collaboration with Taveau and Menge to prepare and study a large number of new cholin and analogous compounds (1), (8), (9), (10), (11), (12).

Acetyl-cholin was but one of nearly a hundred compounds (none of which at that time had been studied pharmacologically and most of which had not previously been made) tested by Taveau and myself and my physiological investigations were necessarily of a preliminary nature and I (incorrectly as I now know) attributed the fall of blood pressure resulting from acetyl-cholin largely to an action upon the heart ("negative inotropic action"). I based this preliminary conclusion upon the following experiments: comparatively large doses cause a marked slowing of the heart evidently from a stimulation of vagus endings; concentrations of acetyl-cholin which had a pronounced weakening effect upon the frog's heart had no effect upon the outflow when perfused through the vessels of the frog; with the animals (dogs) and anaesthetics employed, the myocardiograph showed a marked weakening of the auricle and definite inhibitory changes in the ventricle (which will be described later) when amounts of acetyl-cholin having a minimal effect upon the blood pressure and no effect upon the heart rate, were injected; in two or three experiments in which the *hind*leg of the animal was placed in a plethysmograph acetyl-cholin caused sometimes no change in the volume, sometimes a diminution (evidently passive; see figs. 6 and 14, pp. 206 and 215) or a dilatation which occurred after the blood pressure had returned to normal and which might possibly have been attributed to a rise of vena cava pressure or interpreted as "a reaction to diminished tension" (14); in what seemed to be satisfactory perfusion experiments upon three rabbit ears acetyl-cholin either had no effect on the outflow or caused a diminution (vasoconstriction). Similar results have frequently since been obtained but I realize that they are not sufficient to disprove the contention of Dale that vasodilation is a large factor in the fall of blood pressure from acetyl-cholin.

Another fact which inclined me to the view that the fall of blood pressure was of cardiac origin was the observation made by Taveau and myself in 1906 that the fall was prevented by small amounts of atropine. No one had at that time, so far as I am aware, shown that atropine has a paralyzing action upon vasodilator nerves; in fact it had long been a common physiological demonstration that atropine does not paralyze the most frequently studied of these nerves (chorda tympani). And, of course, it has long been known that atropine does not prevent the action of drugs (nitrites, for example) which are believed to act directly upon the muscle of the vessels.

On the other hand, as is well known, atropine does paralyze all of

the endings of the inhibitory nerves to the heart and it abolishes all of the effects of acetyl-cholin upon the heart; there is not, however, as was shown in one of the earlier papers, a strict parallelism between the action of atropine and of various members of the atropine series in paralyzing the cardio-inhibitory nerves and their action in preventing the fall of blood pressure from acetyl-cholin. I had also observed that acetyl-cholin, and especially some of its homologues, has a stimulating action upon other organs innervated by the parasympathetic nervous system (salivary glands, intestines and eye). Hence the hypothesis that the blood pressure lowering action of acetyl-cholin was but a part of its stimulating action upon the parasympathetic nervous system seemed reasonable; and as the only part of this system the stimulation of which could be expected to cause a fall of blood pressure and which is also paralyzed by small doses of atropine is the cardio-inhibitory mechanism, I was led to the view that this was probably the explanation.

It was, however, easy to confirm the work of Dale that acetyl-cholin does have a pronounced vasodilator action, but there were a number of points which seemed worthy of further investigation. Thus the question, through what mechanism does the drug act, was unanswered. There were, as already intimated, good reasons for believing that the vasodilation was not due to any considerable degree to an action upon the endings of known "parasympathetic vasodilators;" the possibility that it was exerted through the posterior root or the so-called "sympathetic" vasodilators remained to be considered. Also the questions arose whether the mechanism through which acetyl-cholin acts (which is by far the most powerful vasodilator reaction known) is involved in the action of other drugs or in that of the depressor and other nerves causing reflex changes in the blood pressure. These and some other questions will be considered in a subsequent communication. It seemed desirable to first determine more fully in what organs, or vascular areas, the acetyl-cholin vasodilation occurs; this is the chief purpose of the present communication.

THE VASCULAR AREAS INVOLVED IN THE VASODILATION CAUSED BY ACETYL-CHOLIN

The only author who has discussed this subject is Dale; reference will be made to his results in connection with individual organs.

Ligation of arteries to various areas. Before considering the action

of acetyl-cholin[2] upon individual organs brief reference may be made to the results of a few experiments in which the effect of acetyl-cholin upon the blood pressure was determined after the elimination of extensive vascular areas by the ligation of their arteries. The results of such experiments were obviously complicated by the fact that the drug reached the remaining tissues in greater concentration after the elimination of large vascular areas. Thus, with rather high dosage, slowing of the heart (action upon the terminations of the cardio-inhibitory nerves) was more marked after the ligation of a number of arteries.

When the abdominal aorta was clamped just below the diaphragm the absolute fall of blood pressure from a given dose of acetyl-cholin injected into the jugular vein was greatly increased; doses which had previously been ineffective became effective (exps. 349, 350). The percentile fall was usually but not always increased also. Such results show that the splanchnic area is not essential for the vasodilator action of acetyl-cholin. The results were different with nitroglycerin: after clamping the abdominal aorta not only the percentile but the absolute fall was less (exp. 350). These experiments suggest that in the fall of blood pressure from acetyl-cholin the splanchnic area is involved to a less extent than it is in the fall of pressure from nitroglycerin.

Ligation of the main vessels to the splanchnic area (the coeliac axis, the superior and inferior mesenteric and, in some cases, the renal arteries) increased both the absolute and usually the percentile fall of blood pressure (exps. 363, 432, 435, 478, 480, 488); the fall from nitroglycerin was diminished (exp. 480). Ligation of the carotids, subclavians and abdominal aorta (just above the iliacs) diminished both the absolute and percentile fall of blood pressure from acetyl-cholin (exp. 432). Such experiments at least suggest that in the fall of blood pressure from acetyl-cholin the "peripheral" blood-vessels (i.e., those of the skin and muscles) are involved to a greater degree than are those of the "splanchnic" area. It may be mentioned incidentall that (confirmatory of Hartman (15)) in some of these experiments doses of epinephrin which had caused a rise of pressure before the splanchnic arteries were ligated had no effect or caused a fall after their ligation; that doses which had caused a fall of pressure caused a rise after ligation of the "peripheral" arteries. On the other hand, at times, with

[2] By "action of acetyl-cholin" I refer in this paper, unless otherwise stated, to the vasodilator action of small doses which is prevented by atropine; the drug has, under certain conditions, vasoconstrictor and other vascular actions also.

large doses of epinephrin ligation of the coeliac axis increased the rise of pressure; this probably resulted from the increased concentration of the epinephrin in the restricted vascular area (exp. 488).

A number of organs were next examined to determine whether vasodilation occurred in them after the administration of acetyl-cholin. The methods consisted in the use of plethysmographs, the recording of the outflow from a vein and the perfusion of isolated organs with Ringer's solution containing acetyl-cholin. Air plethysmographs were used; the recording apparatus was either a tambour or a form of water manometer the distal limb of which was somewhat enlarged and in which was a float moving in a layer of liquid petrolatum, which transmitted the volume changes to a delicate lever. The apparatus was somewhat similar to that recently described by Hoskins, Gunning and Berry (16). A record of the blood pressure was always taken in the plethysmograph and venous outflow experiments; this often helped in determining whether a given change in volume or outflow was passive or was due to active changes in the vascular areas. In many cases, however, the blood pressure record and the examination of a single organ were not sufficient; I have frequently seen a pronounced change in the volume of an organ or in the venous outflow from it without any change in the blood pressure, but when another organ was simultaneously examined it was found that this was undergoing changes the reverse of those in the other organ; that is, the dilatation or constriction in one area was so nearly equalized by the opposite changes elsewhere that the general blood pressure was unchanged. Accordingly two organs were usually examined at the same time as well as the blood pressure; even then, however, the results of an experiment were sometimes difficult to interpret.

The figures for the doses of acetyl-cholin given in the protocols of the experiments are often far from accurate; they are almost invariably far (often many times) too great. Acetyl-cholin undergoes rather rapid deterioration both in solution and in the dry state. As in most of these experiments I was not especially interested in quantitative results I usually used the same preparation for several days although I knew it to have lost much of its activity; control experiments, however, showed that the changes were purely quantitative, not qualitative, in character. Unless otherwise stated the injections were made intravenously, usually into a saphenous vein.

Limbs; skin; muscle. Dale found, by the plethysmograph method, a dilatation of the cat's leg to result from the intravenous injection of

acetyl-cholin; he does not state whether he considered the dilatation to occur in the skin or muscle or both.

Before describing the effects of acetyl-cholin upon the volume of the limbs, as determined by the plethysmograph, a word may be said as to the effect of this drug upon the vena cava pressure; an objection sometimes made to the interpretation of plethysmograph records is

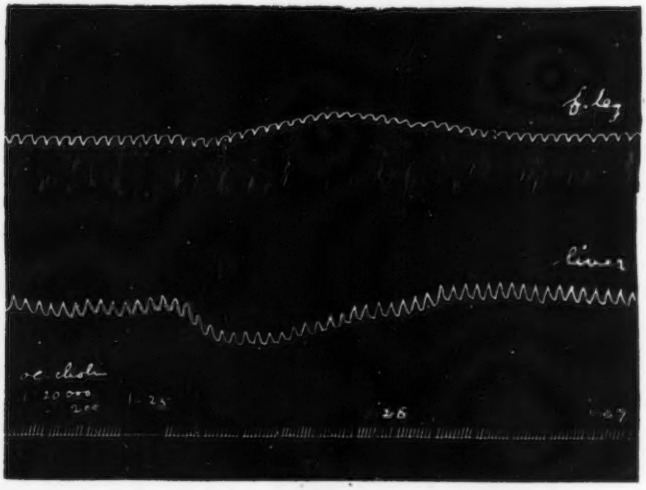

Fig. 2. Experiment 481. Bitch, 6.7 K; morphine; ether; curare; vagi cut. Plethysmograph records from foreleg (above) and left lobe of liver (below). Up = expansion. At *1–25* acetyl-cholin (< 0.1 mg.) injected into saphenous vein. Blood pressure fell 32 mm. Hg.; 0.2 mgm. nitroglycerin caused almost identical effects upon the blood pressure and the volume of the leg and liver; 2 mgm. of atropine sulphate prevented the action of acetyl-cholin but not that of nitroglycerin.

that a rise of vena cava pressure (caused perhaps by an interference with the return of the blood to the heart by some change in the latter) may lead to a passive dilatation of the limbs. However, a number of determinations of the vena cava pressure (made by connecting the central end of a renal vein with a water manometer) showed only a fall when acetyl-cholin was injected intravenously. This fall of vena cava pressure doubtless resulted from an increase in the total vascular area caused by the vasodilator action of acetyl-cholin.

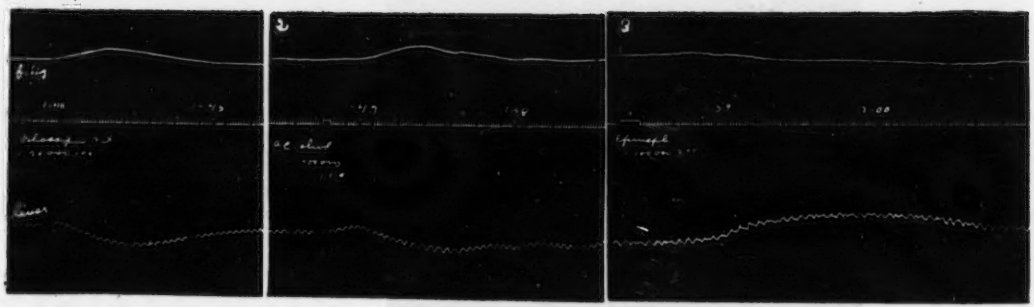

Fig. 3. Experiment 488. Cat, 3.4 K; paraldehyde; vagi cut. Plethysmograph records of foreleg (above) and left lobe of liver (below). Up = expansion. (1) *1–44*, 0.05 mgm. pilocarpine injected into saphenous vein; blood pressure fell 29 mm. Hg. (from 83 to 54). (2) *1–47–*, 0.0025 mgm. acetyl-cholin; blood pressure fell 22 mm. Hg. (71 to 49). When a clamp was placed on the coeliac axis acetyl-cholin caused a greater fall of blood pressure and about the same change in the leg but had no effect on the liver volume. (3) *1–58+*, 0.02 mgm. epinephrin, blood pressure rose 29 mm. (57 to 86).

The cat had an abscess on the nose and had received an overdose of paraldehyde; the blood pressure fell from the beginning.

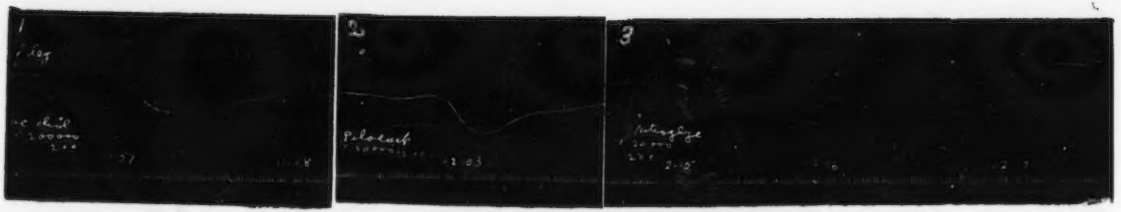

Fig. 4. Experiment 496. Bitch, 8.2 K; morphine; ether. Plethysmograph records from foreleg; down = expansion. (1) *11–56* + < 0.01 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell from 74 to 52 mm. Hg. (2) *12–02* +, 0.05 mgm. pilocarpine hydrochloride; blood pressure fell from 77 to 56 mm. Hg. (3) *12*–15, 0.1 mgm. nitroglycerin; blood pressure fell from 69 to 48 mm. Hg.

Fig. 5. Experiment 471. Cat, 1.88 K; paraldehyde. Plethysmograph records from nasal cavity (above) and foreleg (below): up = expansion. (1) *11–48*, epinephrin 0.01 mgm. into saphenous vein; blood pressure fell 18 mm. (102 to 84). (2) *12–07*, 0.002 mgm. acetyl-cholin, blood pressure fell from 96 to 48 mm. Hg.; 9 mgm. atropine was now injected. (3) *1–38*+, 5 mgm. acetyl-cholin; blood pressure rose from 44 to 78 mm. Hg. (4) *2–02* 0.0025 mgm. epinephrin; blood pressure rose 23 mm.

I invariably found a dilatation when the foreleg of an animal (cat, rabbit, dog) was placed in a plethysmograph and acetyl-cholin, sufficient to cause a fall of blood pressure, was injected. Illustrations of this effect are shown in figures 2 to 7 and 19 (exps. 481, 488, 496, 471, 477, 479, 468). This result was obtained although the blood pressure might be very low (30 mm. Hg., for example). It was also often obtained with doses of acetyl-cholin sufficiently large to cause slowing of the heart; in this case the dilatation was often preceded by slight (passive) contraction (exp. 419). Acetyl-cholin caused an expansion

Fig. 6. Experiment 477. Bitch 8.2 K; morphine; ether. Plethysmograph on right hindleg (above) and right foreleg (below); up = expansion. At 2-47+, 0.2 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell from 106 to 60 mm. Hg.

of the foreleg in experiments in which stimulation of the depressor caused only a (passive) diminution (exp. 423).

When the dose of acetyl-cholin was small, a small dose of atropine abolished the effect upon the limb volume as well as that upon the blood pressure. After atropine, however, a much larger dose of acetyl-cholin caused the leg to expand; this often occurred when there was little or no fall of blood pressure. With still larger doses (and after much atropine) there was a rise of blood pressure or a fall followed by a rise and usually an expansion of the leg; the latter may have been largely passive, however, for with these large doses the curves were

very similar to those following the injection of epinephrin (fig. 5; exp. 471).

The effect of acetyl-cholin upon the volume of the hind limb was usually less marked than that upon the foreleg (fig. 6, exp. 477). Sometimes there was no change although there was a marked fall of blood pressure; not infrequently there was a diminution (evidently passive) although this might be followed by an expansion (fig. 14, exp. 480); frequently there was an expansion which began only when the blood pressure had returned to normal. Sometimes, however,

Fig. 7. Experiment 479. Dog, 6.4 K; morphine; ether, vagi cut. Right forepaw removed, leg skinned and in plethysmograph (below). Left foreleg in plethysmograph (above). Up = expansion. *1–19* +, 0.05 mgm. acetyl-cholin into saphenous vein; the blood pressure fell from 106 to 53 mm. Hg. The effects of nitroglycerin were similar.

there was an expansion which began during the fall of blood pressure (fig. 8, exp. 484). When acetyl-cholin was injected, peripherally, into the femoral artery there was a prompt and marked expansion of the limb (fig. 8); a much smaller dose sufficed to cause a given effect when administered in this way than when injected intravenously. There seems to be no reason for supposing that the vessels of the posterior extremity are less sensitive to acetyl-cholin than are those of the anterior extremities; the smaller reaction of the former probably results from the drug reaching them in a more dilute solution and after dilatation has commenced in areas nearer the heart. It is stated that

amyl nitrite has less effect upon the volume of the foot than upon that of the hand; a similar explanation may hold here also. In experiments in which there was a diminution of the volume of the leg the pulse waves often became more prominent, indicating a relaxation of the vessel walls.

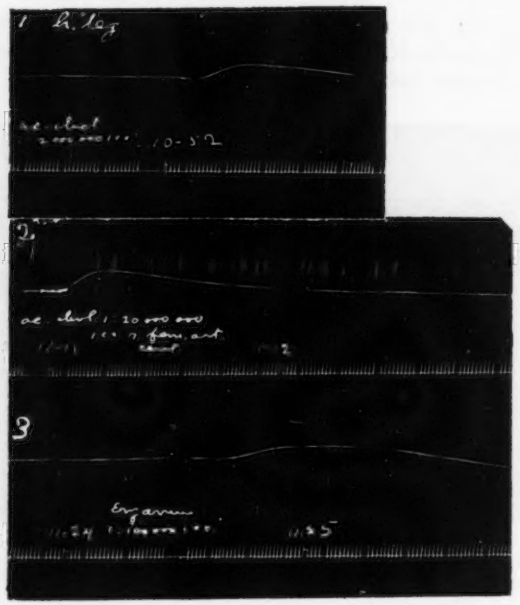

Fig. 8. Experiment 484. Cat, 2.77 K; paraldehyde. Plethysmograph record from left hindleg. Up = expansion. (1) *10–52*, 0.0005 mgm. acetyl-cholin injected into right saphenous vein; blood pressure fell from 133 to 100 mm. Hg. (2) *11–11*, 0.00005 mgm. acetyl-cholin (in 1 cc. normal saline) injected centrally into right femoral artery near its origin; blood pressure fell from 129 to 124 mm. Hg; a control injection of 1 cc. normal saline had no effect upon the volume of the limb. (3) *11–24* +, 0.01 mgm. histamin ("ergamine") intravenously; blood pressure fell from 121 to 84 mm. Hg.

In order to determine whether the dilatation in the limbs resulted chiefly from an effect upon the muscles or upon the skin (and its appendages) the experiment, the results of which are shown in figure 7, (exp. 479), was performed; the changes in the muscle were slight consisting usually of a slight diminution of volume (doubtless passive)

followed by a slight expansion. As the volume of tissue in the two plethsymographs was about equal it would seem that the greater part of the dilatation occurs in the skin or its appendages.

Epinephrin, causing a slight rise followed by a slight fall of blood pressure, caused in this experiment a slight expansion followed by a long continued contraction of the normal leg and only an expansion of the skinned leg; this is in agreement with the results of Hoskins, Gunning and Berry (16). In view of the important deduction these authors drew from their experiments, namely, that epinephrin causes an active dilatation of the vessels of the muscles, attention may be called to the fact that the contraction of the intact limb often continued long after the blood pressure had returned to normal; this seemed also to be the case in the tracing shown in figure 5 of these authors' paper. This continued contraction would seem to be a sufficient explanation of the expansion of the skinned leg which also continued after the blood pressure had returned to normal; hence these authors' statement that "a persistence of the limb expansion after the blood pressure had returned to normal indicates that the dilatation was not a passive process" is not a very strong argument for their view that epinephrin causes an active dilatation of the vessels of the muscles. I infer, however, that these authors obtained an expansion of the skinned leg with depressor doses of epinephrin; it is difficult to see how such a result could be explained except on the basis of active vasodilation in the muscles.

The vasodilator action of acetyl-cholin upon the blood vessels of the limbs (cats) was also shown by recording the drops of blood from veins; the coagulability of the blood was diminished by the injection of hirudin, or the defibrination of the blood and by the use of paraffined cannulas. An illustration of this effect is shown in figure 9 (exp. 483). The increased outflow was most marked toward the end of and after the injection; it often continued for some time after the blood pressure had returned to normal.

The effect of acetyl-cholin upon the outflow from muscle veins was determined in a number of cases (fig. 9); often there was no effect or a very slight, brief increase. Frequently there was a distinct increase for a few seconds, followed by a decrease. In one experiment the outflow from a vein coming from some of the extrinsic muscles of the larynx was determined; there was a marked increase but it was noticed soon afterwards that the animal was making swallowing movements and this may have accounted for the increased outflow.

Although the results of the experiments on the venous outflow from muscles were not entirely conclusive (especially as they were made chiefly upon muscles of the posterior extremities) we certainly seem justified in concluding that dilatation of the vessels of the muscles

can have at most only a minor part in causing the acetyl-cholin fall of blood pressure. The contrast between the constant, prompt and great increase in the outflow from the superficial veins and the, at most, slight and inconstant increase from the muscle veins was very striking.

In a number of these experiments I also tested the effects of epinephrin upon the outflow from superficial and muscle veins: there was a marked decrease in the former and an increase in the latter. The doses injected caused a rise of blood pressure although this was sometimes only a few (e.g., 6) millimeters Hg. This constriction of the superficial vessels was so marked and prolonged that the increased outflow from the muscle could readily be explained as merely a

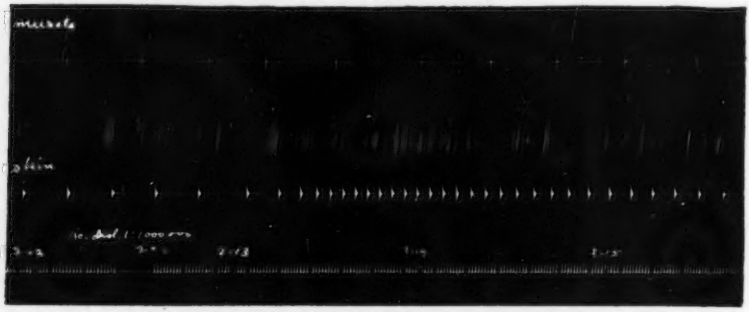

Fig. 9. Experiment 483. Cat, 3.25 K; paraldehyde; hirudin. Drops of blood from muscle branch of left femoral vein (above) and from superficial vein of left forepaw (below). At *2–12* +, 0.002 mgm. acetyl-cholin injected into right saphenous vein; the blood pressure fell 17 mm. Hg.

passive shifting of the blood from one region to another; in other words, they did not necessarily indicate that epinephrin had a dilator action upon the muscle vessels. In one experiment, with a depressor dose of epinephrin, there was a marked increase in the outflow from the muscle but as the latter was occasionally twitching I did not consider the experiment of value. Hoskins, Gunning, and Berry (16), however, found an increased outflow from muscle veins after depressor doses of epinephrin but it may perhaps be questioned whether it is permissible to draw such far-reaching conclusions as these authors do (that epinephrin "exercises a selective vasodilator effect in skeletal muscle;" that the reduced arterial pressure, from depressor doses of epinephrin, is probably due very largely to the augmented outflow from the vessels of the muscles, etc.) from the type of experiments reported. Moreover, if vasodilation in the muscles is such an important action of epinephrin as these authors consider it to be it seems remarkable that it does not, at times at least, occur to such an extent as to lead to a fall of blood pressure in the rabbit; a depressor action of epinephrin does

not seem to have been obtained in this animal even after the pressor action had been prevented by ergotoxine (17) and the same was found for the fowl and the pig (18). Perhaps these doubts are not well founded but in view of the importance of the subject, involving as it does a vasodilator mechanism in the muscles not generally recognized, all of the evidence should be critically examined. It must be admitted, however, that this is the only probable explanation offered for the reported observations.

I may add that, with possibly one exception, I did not observe diminution in the muscle outflow even with doses of epinephrin which caused a doubling of the blood pressure; Gunning (19) has reported such a diminution from "massive" doses of epinephrin but his doses were apparently much greater than those I used. All of my experiments on venous outflow were performed upon cats, whereas Gunning's were performed upon dogs.

Perfusion of isolated muscles (dog, cat) with Ringer solution containing varying amounts of acetyl-cholin showed in one case a slightly increased outflow; in another, weak solutions had no effect but a relatively strong solution caused a marked diminution in outflow. (In some of these experiments I also tried the effects of epinephrin; like others I obtained no indication of a vasodilator action.)

The increased outflow from the superficial veins of the paw following the injection of acetyl-cholin, described above, may have resulted from a dilatation of the cutaneous vessels or of the vessels of the glands of the paws. Hence the outflow was determined from cutaneous vessels on the abdomen and from similar vessels in the legs after removal of the paws; acetyl-cholin caused a marked increase in the outflow, comparable with that from the paw. Areas of skin from the abdomen of cats and rabbits were perfused, some time after the death of the animals, with Ringer solution; a small dose of acetyl-cholin caused a distinct but not very marked increase in the outflow whereas large doses caused a marked diminution.

The effects of acetyl-cholin upon the vessels of the limbs, skin and muscle may be summarized by saying that this drug causes a great dilatation of the limbs and that the dilatation occurs chiefly in the cutaneous vessels.

Ear. Dale found a vasodilation from acetyl-cholin in a rabbit ear perfused with Ringer solution. I have often obtained the same effect. In the first three experiments I obtained no vasodilation; on looking over the records I find that these experiments were performed as follows: the ears were removed under ether anaesthesia, the cannulas were inserted and the perfusion and the injections of acetyl-cholin begun as quickly as possible. Increasing doses of acetyl-cholin were

injected at short intervals; there was no effect except with large doses, and then only a diminished outflow. In some of the later experiments, performed in exactly the same manner, the first injection of acetyl-cholin caused a marked increase in the outflow, but in others there was at first no effect and small doses of sodium nitrite also had little effect; but after some time the outflow increased spontaneously

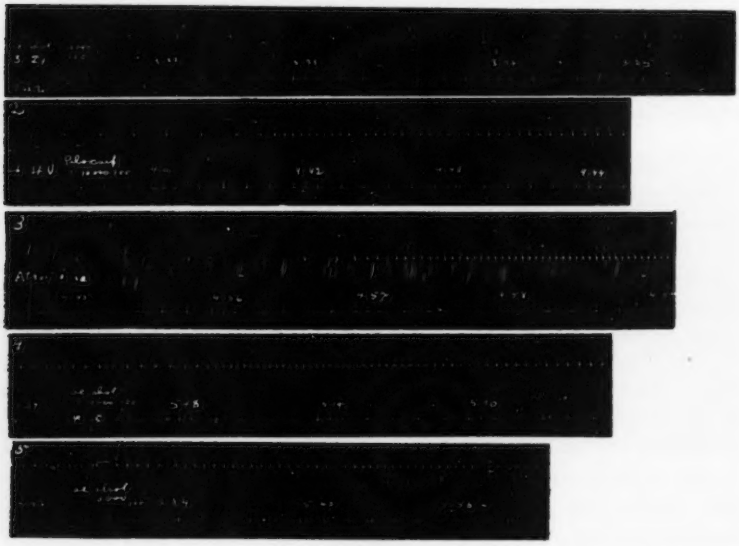

Fig. 10. Experiment 494. Drops from perfused rabbit ear; time in 10 seconds and minutes. The amounts of acetyl-cholin were far less than those indicated. (1) *3–21*, < 0.1 mgm. acetyl-cholin injected; (2) *4–40* +, 0.1 mgm. pilocarpine (3) 2 mgm. atropine sulphate injected at *4–54*. (4) *5–17* + same dose of acetyl-cholin as in (1); the outflow had diminished at *5–21*. Between (4) and (5) 6 mgm. atropine injected. (5) *5–53* + same dose of acetyl-cholin as at (1) and (3). Ten times the amount of acetyl-cholin caused a slight acceleration of the outflow; fifty times a slight slowing.

and now acetyl-cholin was very active. It seems probable that in some cases the vessels are in such a condition of tonicity (perhaps as a result of irritation, change of temperature, etc.) that acetyl-cholin and also small amounts of sodium nitrite are unable to overcome it; later, as the spasmodic condition passes off, the vessels readily dilate under the influence of acetyl-cholin. A small dose of atropine sulphate

lessens this tonicity at once so that the vessels respond to acetyl-cholin; larger doses of atropine abolish the dilating action of acetyl-cholin. Figure 10 (exp. 494) illustrates some of these points. Acetyl-cholin was also frequently more active after small amounts of sodium nitrite had been perfused.

As stated in a previous paper acetyl-cholin in relatively extremely large doses causes a constriction of the vessels of the rabbit ear; I have shown above that it has the same action on cutaneous vessels. Dale found large doses of acetyl-cholin to cause a rise of blood pressure after atropine; I had observed the same but had misinterpreted it. Dale found that after a dose of nicotine sufficient to paralyze the ganglia of the involuntary nervous system acetyl-cholin no longer caused a rise of blood pressure; he attributed the rise of pressure observed before nicotine to a nicotine-like action of the acetyl-cholin (an action also shown by cholin itself but which had been overlooked by those who

Fig. 11. Experiment 497. Drops from perfused rabbit ear; time in 10 seconds and minutes. At *7–01*, 20 mgm. nicotine had been injected; this caused a long continued constriction. *7–46*, 5 mgm. acetyl-cholin injected.

had discussed whether the action of cholin was "depressor" or "pressor"). Although the rise of blood pressure from acetyl-cholin, in the intact animal, is doubtless largely due to this nicotine-like action the experiments on the perfused rabbit ear and cutaneous vessels show that acetyl-cholin also acts peripherally (i.e., beyond the ganglia cells) to cause a vasoconstriction. Atropine seems to be without action in either diminishing or intensifying this effect. Figure 11 (exp. 497) is of interest as showing that this peripheral vaso-constrictor action of acetyl-cholin is not prevented by the previous perfusion of nicotine; the antagonism between the action of nicotine and the pressor action of acetyl-cholin does not seem to extend to the peripheral action of the latter. Similar results were obtained after the perfusion of both atropine and nicotine.

The dilator action of acetyl-cholin upon the rabbit ear was also shown by plethysmographic records (fig. 12, exp. 465; fig. 13, exp. 497).

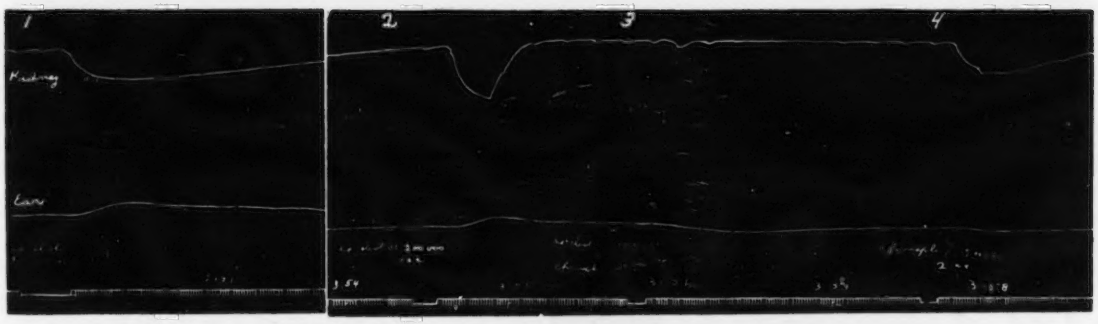

Fig. 12. Experiment 465. Rabbit, 2.05 K; chloral hydrate and ethyl carbamate; vagi cut. Plethysmograph records of left kidney (above) and right ear (below). Up = expansion. Time in seconds, 10 seconds and minutes (1) *2–20* 0.002 mgm. acetyl-cholin into femoral vein; duration of injection 20 seconds; blood pressure fell 39 mm. Hg. (from 93 to 54). Small dose of atropine now injected. (2) *3–54* +, 0.005 mgm. acetyl-cholin injected; blood pressure fell 51 mm. (3) *3–55* +, 0.01 mgm. acetyl-cholin and 0.02 mgm. epinephrin (mixed) injected; blood pressure fell from 100 to 81 mm., then rose to 112 mm. (4) *3–58* −, 0.01 mgm. epinephrin; blood pressure rose 26 mm.

Fig. 13. Experiment 497. Rabbit, 1.7 K; ethyl carbamate; ether. Plethysmograph record of right ear. Down = expansion. (1) *11–47*, 0.5 mgm. pilocarpine hydrochlor. into saphena vein; blood pressure fell from 102 to 71 mm. Hg.; heart slowed from 42 to 31 in 10 seconds. (2) *11–49* +, < 0.0025 mgm. acetyl-cholin; blood pressure fell from 104 to 74 mm. (3) *1–05*, 0.005 mgm. epinephrin; blood pressure rose 26 mm.

(The usual effect of epinephrin upon the ear was to cause a constriction; fig. 13, exp. 497.)

Penis. Plethysmograph records showed a slight expansion of the penis after acetyl-cholin or a contraction (evidently passive). After ligation of the coeliac axis, the mesenteric and renal arteries, acetyl-cholin caused a pronounced expansion of the penis; this was abolished by atropine (fig. 14, exp. 480).

Fig. 14. Experiment 480. Dog, 7.1 K; morphine; ether. The coeliac axis, the renal and mesenteric arteries had been ligated. Plethysmograph records of hindleg (above) and penis (below). Up = expansion. *1–19* +, 0.02 mgm. acetyl-cholin into vein of forepaw; blood pressure fell from 104 to 46 mm. Hg. After 2 mgm. atropine sulphate 0.5 mgm. acetyl-cholin had no effect; nitroglycerin caused a slight expansion of leg and penis; a small amount of epinephrin caused a contraction of the leg and a very slight contraction of the penis; a larger amount caused a marked contraction of the leg and a slight (passive) expansion of the penis.

Submaxillary gland. Acetyl-cholin, injected intravenously, caused a brief increase in the outflow of blood from the submaxillary gland (fig. 15, exp. 504); a more prolonged acceleration of the outflow could be obtained by a slow intravenous infusion or by applying a drop of a rather strong solution (e.g., 1:1000) to a muscle. The vasodilator action of acetyl-cholin upon the vessels of the submaxillary gland was abolished with great ease by atropine. Thus (exp. 461), 0.0002 mgm. acetyl-cholin caused a pronounced but brief increase in the outflow

and a marked fall of blood pressure; after 0.05 mgm. atropine sulphate, 0.001 mgm. acetyl-cholin had no effect on the outflow but caused a slight fall of blood pressure. After 2 mgm. of atropine 0.1 mgm. of acetyl-cholin caused a slight fall of blood pressure but had no effect on the venous outflow. After a further injection of 5 mgm. atropine, 2 mgm. acetyl-cholin caused a very slight fall of blood pressure and a very slight diminution of the outflow. In this and similar experiments sti ulat'on of the chorda tympani continued to cause a marked increase in the venous outflow. No secretion of saliva (as observed in a cannula in the gland duct) resulted from these rapid intravenous injections of acetyl-cholin. In one experiment the venous outflow from the submaxillary gland was reduced by about one-half by stimulation of the cervical sympathetic; during the stimulation acetyl-cholin was

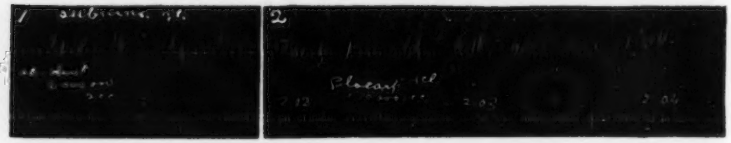

Fig. 15. Experiment 504. Cat, 3.2 K; ethyl carbamate; hirudin. Drops of blood from vein of submaxillary gland. Time in seconds, 10 seconds and minutes. (1) *12*, < 0.001 mgm. (old preparation) of acetyl-cholin into saphenous vein; blood pressure fell from 100 to 59 mm. Hg. (2) *12–02+*, 0.1 mgm. pilocarpine; blood pressure fell from 90 to 52 mm. The fall of blood pressure from the pilocarpine was slightly more prolonged than that from the acetyl-cholin.

injected intravenously and the outflow increased to almost normal for about ten seconds.

Thyroid. The outflow from a vein on the anterior surface of the trachea (cat), formed chiefly by the union of veins from the thyroid, was slightly diminished (passively?) by doses of acetyl-cholin causing a moderate fall of blood pressure. Applied, on filter paper, to the surface of the thyroid there was, according to the dose a slightly increased outflow or a diminished outflow (vasoconstriction). (Both pressor and depressor doses of epinephrin caused a marked diminution of the outflow—confirmatory of Gunning (20); that the diminished outflow from depressor doses was not entirely passive was indicated by the fact that the diminished outflow continued longer than the fall of blood pressure.)

Nasal mucosa. The nasal mucosa, examined by the plethysmograph method Tschalussow (Chalussov) (21), uniformly showed a contraction after the injection of acetyl-cholin although this was often preceded by an expansion (fig. 5, exp. 471; fig. 16, exp. 469). With

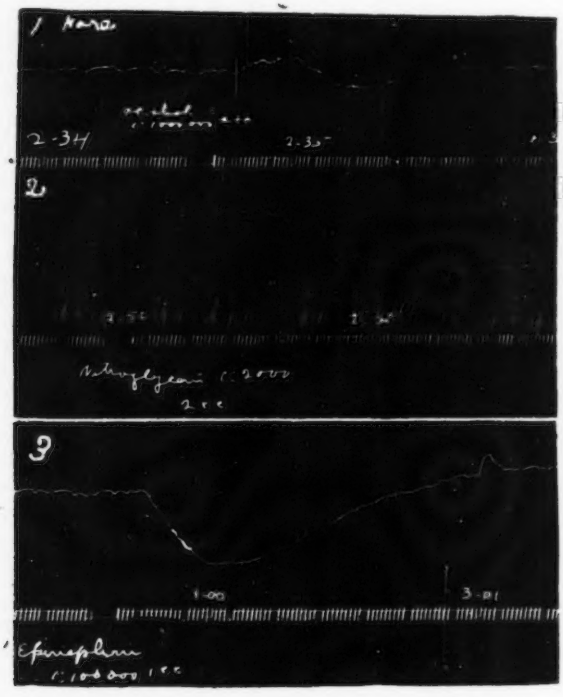

Fig. 16. Experiment 469. Dog, 5.84 K; morphine; chlorbutanol; vagi cut. Plethysmograph records from nose. Up = expansion. (1) *2–34+*, 0.002 mgm. acetyl-cholin into right saphenous vein; blood pressure fell 23 mm. (2) *2–50*, 1 mgm. nitroglycerin; blood pressure fell 26 mm. (3) *3–00–*, 0.01 mgm. epinephrin; blood pressure rose 39 mm. Control injections of normal saline had no effect.

very small doses of acetyl-cholin (but sufficient to cause a pronounced fall of blood pressure) I consider this diminution of the volume of the nasal mucosa to be a passive effect for it is prevented by atropine; atropine does not prevent, but makes more prominent, the vaso-constrictor

(nicotine-like according to Dale) action of acetyl-cholin. The primary expansion which frequently occurs is due to vasodilation; the pulse waves are usually more distinct (fig. 16).

The difficulty of interpreting with certainty the action of a drug like acetyl-cholin (and the same is true of epinephrin) which has both a vasodilator and a vasoconstrictor action (according to dose, the organ studied and other factors) is well illustrated by the experiment (471) in which the tracings shown in figure 5 were obtained. Figure 5 shows that 0.002 mgm. acetyl-cholin caused a distinct diminution of the volume of the nasal mucosa and a slight increase in the volume of the foreleg; the blood pressure fell 38 mm. Hg. (from 96 to 58 mm.). After 0.5 mgm. atropine sulphate this amount of acetyl-cholin did not have the slightest effect upon the blood pressure or the nasal mucosa or the leg. But 0.1 mgm. acetyl-cholin caused a fall of blood pressure of 12 mm. Hg. a greater diminution in the volume of the nasal mucosa and no change in the volume of the leg (or the heart rate). The diminution in this case was probably due in part to the beginning vasoconstrictor ("nicotine-like") action of the acetyl-cholin (for the fall of blood pressure was less and the contraction of the mucosa greater than above; the fall of blood pressure was probably due to a vasodilatation occurring elsewhere than in the leg. After the further injection of 3.5 mgm. atropine 0.1 mgm. acetyl-cholin had no effect on the blood pressure but there was a diminution of the volume of the nasal mucosa and an expansion of the leg. Standing alone it would be impossible to interpret this result (and similar problems arise with epinephrin); thus it might be supposed *a*, that there was an active constriction in the nasal mucosa and an active dilatation in the leg; or *b*, that there was an active contraction in the mucosa and elsewhere, with a passive expansion of the leg; or *c*, that there was an active dilatation in the leg with a passive contraction in the mucosa. Taken in connection with the results preceding and following this injection, however, the second seems to be the more probable explanation. The further injection of atropine had no effect upon the changes caused by the above dose of acetyl-cholin but larger doses of acetyl-cholin caused a rise of blood pressure, a greater contraction of the mucosa and a greater expansion of the leg. The tracing at *1—38+* in figure 5 shows the effect of 5 mgm. of acetyl-cholin; the blood pressure rose 34 mm. Hg. In this case there seemed to be an active constriction in the nasal mucosa, and doubtless in other organs, which led to the rise of blood pressure and a passive expansion of the leg.

Small doses of epinephrin caused in this experiment a fall of blood pressure, a marked contraction of the nasal mucosa (fig. 5) and no change in the volume of the leg. Standing alone the contraction of the mucosa might be interpreted as a passive result of the fall of blood pressure. With doses causing a rise followed by an equal fall of pressure there was a greater contraction of the mucosa and a slight expansion of the leg. With a purely pressor dose of epinephrin curves almost identical with those from pressor doses of acetyl-cholin were obtained, although the rise of pressure was less after epinephrin (fig. 5). The expansion of the leg might easily have been interpreted as due to an active vasodilatation but considered in connection with the other results it was almost certainly passive, the change in the mucosa being due to an active vasoconstriction.

In this experiment a dose of nitroglycerin causing the same fall of blood-pressure as a dose of acetyl-cholin, caused about the same change in the leg volume but had less effect upon the nasal mucosa.

In an experiment upon a dog (fig. 16, exp. 469), acetyl-cholin caused a slight contraction of the mucosa or a slight expansion followed by a contraction or a slight contraction followed by a longer but slight expansion; nitroglycerin in doses causing an equal or less fall of blood pressure uniformly caused a marked expansion of the nasal mucosa followed by a prolonged (probably passive) contraction. A pressor dose of epinephrin caused a marked contraction followed by a long-continued expansion.

The above experiments indicate that depressor doses of acetyl-cholin have relatively little effect upon the nasal mucosa although in the dog there was at times a distinct dilatation, but this was usually obscured by a passive contraction due to the fall of blood pressure resulting from a greater dilatation elsewhere; the marked tendency of the nasal mucosa to respond passively to changes in blood pressure was emphazised by Fofanow and Tschalussow (Fofanov and Chalussov) (22). These experiments also indicate that the nasal mucosa is relatively less sensitive to acetyl-cholin than it is to nitroglycerin or epinephrin.

Intestines. Dale, using a plethysmograph, found an expansion of the intestine (cat) after acetyl-cholin. My experiments were performed upon rabbits and a dog; they were not very satisfactory. I am not certain that the results were not complicated by contractions of the intestine although Dale found that it required a relatively very large dose of acetyl-cholin, given intravenously, to affect the intestinal movements even for a brief period. In the rabbit there was sometimes a slight contraction, sometimes a contraction followed by a more prolonged expansion (fig. 17, exp. 456). The latter type of curve was also obtained in a dog (fig. 18, exp. 469). (The usual effect of epinephrin in pressor doses in these experiments was to cause an expansion of the intestine; whether this was passive or active I do not know.)

In the only experiment in which the outflow from a vein from the small intestine (cat) was determined, acetyl-cholin caused a diminished outflow (probably passive) with a pronounced fall of blood pressure.

A large loop of the isolated small intestine of a rabbit was perfused with Ringer solution; acetyl-cholin caused intestinal movements with a diminution of the outflow After atropine the same (small) doses of acetyl-cholin had no effect either upon the movements or outflow. (Sodium nitrite caused an increased outflow.)

Spleen. The effect of acetyl-cholin upon the volume of the spleen, as determined by the plethysmograph, was somewhat variable and sometimes puzzling on account of the development of rhythmical

Fig. 17. Experiment 456. Rabbit, 1.74 K; paraldehyde. Plethysmograph record of loop of small intestine. Up=expansion; 0.5 mgm. atropine sulphate had been given; this had greatly diminished the action of acetyl-cholin upon the blood pressure. At *1–10+*, 0.1 mgm. acetyl-cholin into femoral vein; blood pressure rose, briefly, 8 mm. (probably mechanical effect of injection) and then fell 21 mm. The fall of blood pressure was prolonged, but not as prolonged as the expansion of the intestine.

Fig. 18. Experiment 469. Dog, 5.84 K; morphine; chlorbutanol. Plethysmograph record of loop of small intestine. Up = expansion. *12–42+*, 0.02 mgm. acetyl-cholin into saphenous vein; blood pressure fell 23 mm. Hg.

changes in the volume of the spleen. Sometimes there was a diminution of the volume, apparently passive, during the fall of blood pressure, or a diminution followed by an expansion (fig. 19, exp. 468);

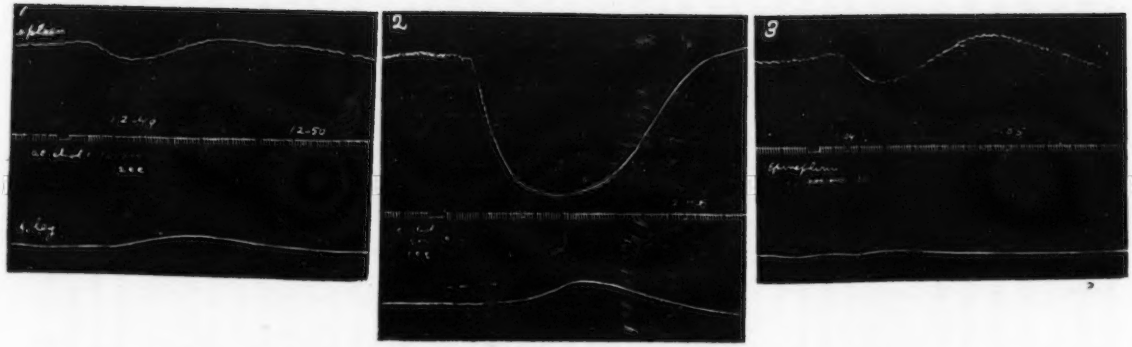

Fig. 19. Experiment 468. Cat, 2.4 K; paraldehyde; vagi cut. Plethysmograph record of spleen (above) and right foreleg (below). Up = expansion. (1) *12–49–*, 0.00002 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell 13 mm. Hg. (2) *2–44+*, 5 mgm. acetyl-cholin; blood pressure fell 6 mm., then rose 13 mm. Before this injection a total of 19.5 atropine had been injected. (3) *1–04–*, 0.001 mgm. epinephrin; blood pressure rose 7 mm.

expansion was often followed by two or more extensive rhythmical variations in volume during which the volume of the spleen was greater than before the injection. I am inclined to think that the records can be interpreted as showing an active vasodilation in the spleen after acetyl-cholin; during the diminution of the volume the vessels may have relaxed but the organ diminished in size as a result of greater dilatation elsewhere (in the skin of the legs, for example).

The experiment (468) in which the tracing in figure 19 was obtained presented many of the problems as regards both acetyl-cholin and epinephrin which were discussed in connection with the experiments on the volume changes in the nasal mucosa. As figure 19 shows, 0.00005 mgm. of acetyl-cholin caused a contraction, followed by a slight expansion, of the spleen and an expansion of the foreleg; the blood pressure fell 13 mm. All of these effects from small doses of acetyl-cholin were completely prevented by 1 mgm. of atropine; 0.01 mgm. acetyl-cholin, however, caused a slight contraction followed by a distinct expansion (complicated, however, by extensive rhythmical changes) of the spleen and a marked expansion of the leg; the blood pressure fell 15 mm. Hg. After 2 mgm. of atropine the above dose of acetyl-cholin (0.01 mgm.), and also 0.1 mgm., had none of these effects but 1 mgm. of acetyl-cholin caused the blood pressure to fall 23 mm., the spleen to contract to a far greater extent than it had previously, although in some cases the fall of blood pressure had been greater (hence the contraction now was probably in part active) and the leg expanded markedly. After a further injection of atropine, acetyl-cholin had either no effect upon the blood pressure or caused an insignificant fall followed by a rise or only a rise; that is, the dilator action of acetyl-cholin had been paralyzed. Doses, however, which caused no change in the blood pressure continued to cause a contraction of the spleen and a dilatation of the leg. With larger (essentially pressor) doses of acetyl-cholin there was a very great contraction of the spleen (evidently not passive, for the blood pressure rose) and a marked expansion of the leg (perhaps passive) (fig. 19).

Small, but weakly pressor doses of epinephrin caused a marked contraction of the spleen usually followed by an equally marked dilatation and a slight dilatation of the leg (fig. 19); similar changes in the spleen were described by Hoskins and Gunning (23) and by Hartman and McPhedran (24). I was not certain whether this after-dilatation of the spleen was to be considered active or passive; it may be that the spleen, on account of its relative proximity to the heart, responds first with an active constriction and then, as other more distant structures are constricted, passively dilates. As the dose of epinephrin was increased only a marked contraction of the spleen and a correspondingly greater dilatation of the leg was obtained; such a result was obtained with doses of epinephrin causing a rise of blood pressure of only 10 mm. Hg. In this case the dilatation of the leg was probably only passive although the volume curve of the leg considered alone, or with the blood pressure curve, might easily have been interpreted as showing an active dilatation of the leg.

Any doubt as to the ability of acetyl-cholin to cause a dilatation of the vessels of the spleen was removed by perfusing the isolated organ with Ringer solution containing the drug: small amounts caused a greater and more prolonged increase in the outflow than occurred in similar experiments on the rabbit ear. The relation to atropine was similar; the latter of itself in small doses caused a marked increase in the outflow and also rendered the vessels more sensitive to acetyl-cholin. After large doses of atropine, acetyl-cholin had no dilator effect. Large doses of acetyl-cholin caused a marked diminution of the outflow. The records of these experiments are so similar to those obtained with the rabbit ear (see figs. 10 and 11) that it is not necessary to reproduce them; the chief difference was that the changes were of longer duration in the case of the spleen. Epinephrin greatly diminished, sodium nitrite increased, the outflow from the spleen.

Liver. Changes in the volume of the left lobe of the liver, after acetyl-cholin, were determined by the plethysmograph method in one dog and two cats. In all cases there was first a diminution in volume (fig. 3, exp. 488; fig. 2, exp. 481; fig. 20, exp. 486). With large doses the contraction was followed by an expansion in the dog. After atropine large (pressor) doses of acetyl-cholin caused only an expansion. After incomplete atropinization comparatively large doses of acetyl-cholin (e.g., 2 mgm.) caused a slight fall followed by a slight rise of blood pressure and a contraction followed by an expansion of the liver (fig. 20).

The diminution in liver volume from small doses of acetyl-cholin was probably passive due to the lowered blood pressure. It apparently resulted from a diminution of the blood supply to the liver through the hepatic artery for it did not occur when this was clamped. Similarly the expansion of the liver from pressor doses of acetyl-cholin after atropine was probably in part at least passive.

The only indication of an active dilatation of the liver observed was the expansion, following a contraction, often seen in the dog after a comparatively large (but still purely depressor) dose of acetyl-cholin (fig. 2); this result was similar to that usually seen in the volume changes of the intestine of the dog. For reasons given below it scarcely seems probable that these changes in the liver volume result from changes in the portal vessels. Hence they may be taken as an indication that acetyl-cholin has a dilator action upon the terminations of the hepatic artery in the liver but that the first effect upon

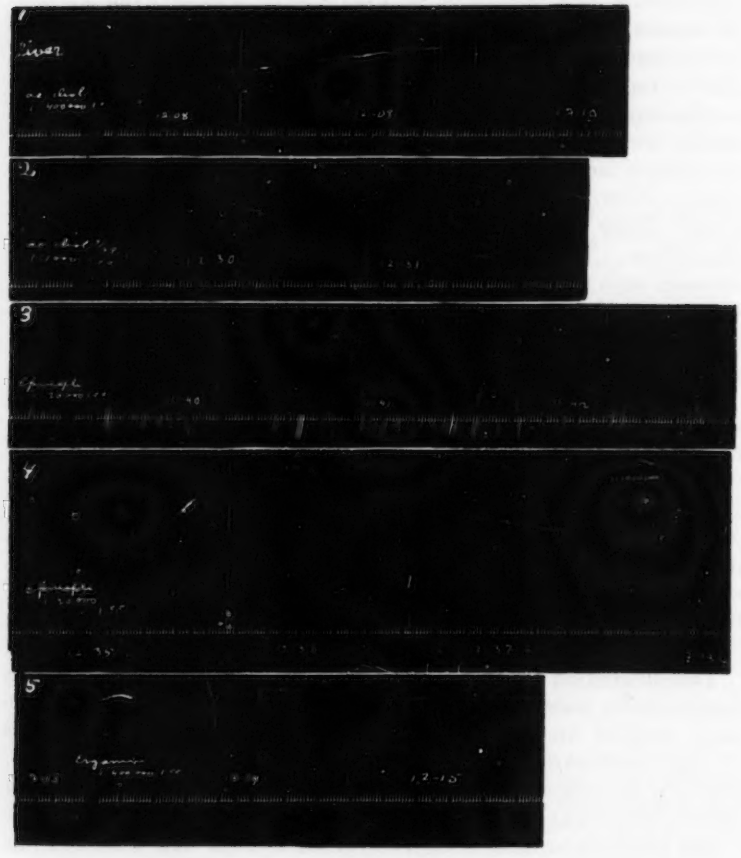

Fig. 20. Experiment 486. Cat, 2.26 K; paraldehyde. Plethysmograph record of left lobe of liver. Up = expansion. (1) *12–07+*, 0.0025 mgm. acetyl-cholin injected into saphenous vein; blood pressure fell 24 mm. Hg. *12–17*, 2 mgm. atropine injected. (2) *12–29+*, 2 mgm. acetyl-cholin; blood pressure fell 9, then rose 9 mm. Hg. (3) *11–39+*, epinephrin 0.05 mgm; blood pressure rose 56 mm. (4) *12–35*, epinephrin 0.05 mgm.; blood pressure rose 68 mm. (5) *12–13*, histamin ("ergamine") 0.0025 mgm.; blood pressure fell 22 mm.

the volume of the liver is a diminution due to a greater vasodilation elsewhere.

The results of the injection of epinephrin upon the liver volume in these experiments are of some interest. Edmunds (25) found the usual effect upon the liver of epinephrin (in pressor doses) to be a diminution of the volume in the case of the dog; in rare cases there was an expansion. The latter result, an expansion, was the usual response in cats; the expansion he attributed to an increased efficiency of the heart. Mautner and Pick (26) report an active contraction of the liver from epinephrin in the dog and cat. In my experiment upon a dog and in one of the experiments on cats, epinephrin (in pressor doses) caused at first only a marked contraction; the latter was as pronounced in the cat (fig. 20) as in the dog. Peptone was injected into the dog; this caused a marked expansion of the liver and a fall of blood pressure which, however, later returned to normal. After the peptone injection epinephrin caused only an expansion of the liver. Peptone was also injected into the cat; there was a contraction of the liver and a fall of blood pressure; the latter remained low. Epinephrin continued to cause a contraction of the liver but after 2 mgm. atropine sulphate, which caused a further fall of blood pressure, epinephrin caused only an expansion (fig. 20). In the second experiment upon a cat the blood pressure fell from the beginning, perhaps from an overdose of paraldehyde; epinephrin caused only an expansion of the liver (fig. 3; exp. 488).

Thus in the experiment on a dog and in one of the experiments on cats the primary effect of epinephrin was to cause a contraction of the liver. Later, after various insults, the liver responded with only an expansion; animals at this time may be considered to have been in a condition somewhat analogous to experimental shock, although the blood pressure in the dog was as high as at the beginning (about 100 mm.) when epinephrin caused only a contraction of the liver. The second cat was in a condition analogous to shock from the beginning. The results in these three experiments were so striking as to suggest the thought: May not one of the features of experimental "shock" be a change in the blood vessels of the liver such that they can no longer respond with a contraction to epinephrin or rather, perhaps, that they are no longer able to offer a resistance to a rise of blood pressure? The expansion of the liver after epinephrin seemed to be passive; it was accompanied by a marked contraction of the leg, and was, in the only experiment (cat) in which it was tested, prevented by clamping the hepatic artery. In the latter case there was not a late expansion of the liver described by Edmunds when the hepatic artery was clamped and ascribed by him to a rise of vena cava pressure.

As stated above there seems no reason for believing that the change in the liver after acetyl-cholin are due to an effect upon the terminations of the portal vein. Another reason for doubting such an effect was the failure to obtain more than at most an extremely slight increase in the outflow from the liver (guinea pig) when this was perfused through the portal vein with Ringer solution containing various amounts of acetyl-cholin; higher concentration caused a diminution of the

outflow but the vasoconstrictor action of large doses of acetyl-cholin seemed to be less marked in the liver than in most of the organs studied.

Epinephrin in this experiment caused only a moderate diminution in the outflow: this confirmatory of Mautner and Pick (26); the vasoconstrictor action was far less marked than in the case of most organs examined. Atropine caused a very slight increase; sodium nitrite, in relatively large doses, caused a considerable increase, followed in one case by a diminution.

In the only experiment (cat) in which it was tested the portal pressure fell with doses of acetyl-cholin causing a marked fall of arterial pressure. This was probably a passive effect for although there is evidence that acetyl-cholin has a dilator action upon the vessels of the intestine this does not seem to be sufficiently great (in some cases at least), in comparison with this action in other areas, to lead to an increased outflow from the intestine and so to a rise of portal pressure; as was stated above there was a diminished outflow from a mesenteric vein in the only case in which this was determined. (In the experiment in which the portal pressure fell after acetyl-cholin, epinephrin caused a slow but pronounced rise.)

Injection of acetyl-cholin into a mesenteric vein. I have assumed in the above that even if acetyl-cholin has a pronounced dilator action upon the terminations of the portal vein this would not be in evidence when the drug is injected into a systemic vein. I have assumed that there would be a considerable destruction of the compound during its relatively slow passage through the vessels of the intestine and that it would reach the liver in too great a dilution to affect this organ. I have performed no experiments (Eck fistula, for example) to test this assumption. The following experiments, however, may be of interest in this connection as showing the effect of passing the drug through the liver. Small doses of acetyl-cholin which, when injected into a systemic vein caused a pronounced fall of blood pressure, had not the slightest effect when injected into a mesenteric vein. In an experiment (cat) in which comparisons were made it was found that to cause an equal fall of blood pressure fully one hundred times as much acetyl-cholin had to be injected into a mesenteric as into a saphena vein. Thus 0.01 mgm. injected into a mesenteric vein caused the blood pressure to fall 31 mm.; 0.0001 mgm. into a saphena vein caused a fall of 58 mm. (Similar but less marked differences were observed in connection with nitroglycerin and, in different experiments, with pressor and depressor doses of epinephrin; but in one case in which a depressor dose of epinephrin was injected the fall of pressure

was greater when it was injected into a mesenteric vein and in another case as the dose was gradually increased from a depressor to a pressor dose the change occurred first from the mesenteric injection, i.e., the same dose injected into the mesenteric vein caused a rise of pressure and when injected into the saphena vein caused a fall.)

Experiments upon the absorption, as judged by the effect upon the blood pressure, of acetyl-cholin when applied to the surface of various organs are of interest in this connection. The experiments were made by applying small squares of filter paper moistened with solutions of acetyl-cholin to various organs; it was determined beforehand how much, by weight, the pieces of paper absorbed. I made no effort to determine how much acetyl-cholin actually reached the circulation. In one experiment when squares of paper moistened with approximately 4 mgm. of a 1 per cent solution of acetyl-cholin were applied for thirty to forty seconds to the surface of various organs, the following results were obtained:

Liver: blood pressure fell 62 mm. Hg. (from 144 to 82).
Stomach: no effect.
Adrenal: blood pressure fell 76 mm. Hg. (from 139 to 63).
Small intestine: (4 and 8 mgm.) no effect.
Liver: blood pressure fell 72 mm. Hg. (from 146 to 74).
Kidney: blood pressure fell 60 mm. Hg. (from 143 to 83).
Spleen: no effect.

Thus the acetyl-cholin was either very imperfectly absorbed from the surface of the small intestine, spleen and stomach or, what is more probable, it was absorbed and rendered inert in its passage through the liver. Acetyl-cholin was absorbed with great ease from the surface of the lung; this is a very convenient method of obtaining a prolonged and fairly uniform lowering of the blood pressure. A small amount, e.g., 0.002 mgm., injected into the trachea also caused a prolonged fall of pressure. Absorption occurred, but to a lesser extent as judged from the effect on the blood pressure, when acetyl-cholin was applied to the conjunctiva and the nasal mucosa; it was very slight from the prepuce. It was well absorbed from the surface of a muscle. Acetyl-cholin injected peripherally into the artery of a limb caused a fairly marked fall of blood pressure: thus 0.0005 mgm. injected peripherally into the femoral artery of a cat caused the arterial (carotid) pressure to fall 20 mm.; the same amount injected into a saphena vein caused the pressure to fall 33 mm.

Kidney. Acetyl-cholin caused only a diminution of the kidney volume as determined by the plethysmograph (fig. 12, exp. 465); this was prevented by a dose of atropine which prevented a fall of blood pressure. The contraction seemed to be a passive effect. The only indication of a dilator action I observed in these experiments is shown in figure 12: when sufficient epinephrin was added to the acetyl-cholin to greatly diminish the fall of blood pressure there was no distinct effect upon the kidney although a smaller (pressor) dose of epinephrin caused a marked contraction of the kidney. A contraction of the kidney would have been expected from the acetyl-cholin and epinephrin mixture if the acetyl-cholin did not have a dilator or antagonistic action to the epinephrin; the fact that a contraction did not occur suggests that the acetyl-cholin prevented in some way the constrictor action of the epinephrin. Perfused through the isolated kidney (rabbit) acetyl-cholin caused a slight increase in the outflow; this was very small in comparison with the effects of sodium nitrite. (The usual effect of epinephrin in pressor doses was, in these experiments, to cause a contraction of the kidney volume; with very large doses, causing a great rise of blood pressure, the contraction was frequently followed by an expansion, evidently passive.)

Lung. A lobe of a lung (cat, rabbit or guinea pig) was perfused with Ringer solution containing acetyl-cholin; in no case was there a distinct increase in the outflow. There was, on the contrary, with large doses, in all three cases a great and very prolonged decrease in the outflow; in some cases the outflow did not return to normal until after thirty minutes. Atropine (at least in the comparatively large doses employed) did not prevent this action.

Atropine itself, which so often causes an increased outflow from perfused organs, had no effect. Sodium nitrite caused in the experiments with the rabbit and guinea pig lungs (it was not tried with the cat lung) first a brief increase (50 to 100 per cent) in the outflow followed by a great and very prolonged diminution. It was sometimes an hour before the outflow returned to the previous rate; the result was strikingly like the vasoconstriction often seen in the perfusion of other organs with epinephrin. The rabbit lung showed the same reactions twenty hours after the first injection. Macht (27) has shown that the nitrites cause a contraction of strips of the pulmonary artery and he cites earlier experimental and clinical work which indicated that these bodies constrict the pulmonary vessels *in vivo;* I do not know of any previous perfusion experiments on this subject.

SUMMARY

It has been shown in the above that acetyl-cholin has an intense vasodilator action on the vessels of the skin and of the ear; the action on the skeletal muscles is slight. It dilates the vessels of the penis, of the submaxillary gland and of the spleen; it seems also to dilate the vessels of the intestines and liver. Only slight evidence of a dilator action was found in the case of the kidney and none in that of the lung. The nasal mucosa seemed relatively less sensitive to the vasodilator action of acetyl-cholin than many other vascular areas. The vasodilation in all of these cases was diminished or prevented by atropine.

As little as 0.000,000,002,4 mgm. acetyl-cholin per K caused a pronounced fall of blood pressure.

Acetyl-cholin injected into the trachea or applied to the surface of the lung, kidney, liver, adrenal and various muscles was very active in causing a fall of blood pressure; similar doses applied to the surface of the stomach, spleen and small intestine had no effect on the blood pressure.

BIBLIOGRAPHY

(1) HUNT AND TAVEAU: Brit. Med. Journ., 1906, ii, 1788.
(2) HUNT: Journ. Pharm. Exper. Therap., 1915, vii, 301.
(3) GUGGENHEIM AND LOEFFLER: Biochem. Zeitschr., 1916, lxxiv, 208; quoted from Chem. Abst., 1916, x, 2754.
(4) FÜHNER: Biochem. Zeitschr., 1916, lxxvii, 408; quoted from Chem. Abst., 1917, xi, 616.
(5) KAUFFMANN AND VORLÄNDER: Ber., 1910, xliii, 2735.
(6) HUNT: This Journal, 1900, iii, 18.
(7) HUNT: This Journal, 1901, v, 6.
(8) HUNT AND TAVEAU: Journ. Pharm. Exper. Therap., 1909, i, 303.
(9) HUNT AND TAVEAU: Bull. 73, Hygienic Laboratory, U. S. Public Health Service, 1911.
(10) MENGE: Journ. Biol. Chem., 1911, x, 399; 1912, xiii, 97.
(11) MENGE: Bull. 96, Hygienic Laboratory, U. S. Public Health Service, 1914.
(12) HUNT: Journ. Pharm. Exper. Therap., 1915, vi, 477.
(13) DALE: Journ. Pharm. Exper. Therap., 1914, vi, 147.
(14) BAYLISS: Journ. Physiol., 1902, xxviii, 220.
(15) HARTMAN: This Journal, 1915, xxxviii, 438.
(16) HOSKINS, GUNNING AND BERRY: This Journal, 1916, xli, 513.
(17) DALE: Journ. Physiol., 1906, xxxiv, 163.
(18) BARGER AND DALE: Biochem. Journ., 1907, ii, 240.
(19) GUNNING: This Journal, 1917, xliii, 395.
(20) GUNNING: This Journal, 1917, xliv, 215.
(21) TSCHALUSSOW: Pflüger's Arch., 1913, cli, 524.

(22) Fofanow and Tschalussow: Pflüger's Arch. 1913, cii, 551.
(23) Hoskins and Gunning: This Journal, 1917, xliii, 298.
(24) Hartman and McPhedran: This Journal, 1917, xliii, 311.
(25) Edmunds: Journ. Pharm. Exper. Therap., 1915, vi, 569.
(26) Mautner and Pick: Münch. Med. Wochenschr., 1915, 1141.
(27) Macht: Journ. Pharm. Exper. Therap., 1914, vi, 13.

VASODILATOR REACTIONS. II

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Received for publication December 7, 1917

1. THE RELATION OF THE ACETYL-CHOLIN VASODILATOR REACTION TO VASOMOTOR NERVES

It has been shown by the work of Dale (1) and also by that reported in the preceding paper that acetyl-cholin causes vasodilation in many organs and that this action is prevented by atropine. It is usually assumed that a drug, the effect of which is prevented by atropine, acts upon "nerve endings" or upon "receptive substances" in connection with nerve endings; moreover, such an antagonism is often interpreted as showing the presence in an organ of a parasympathetic nerve innervation. Hence it was of interest to see if this vasodilator reaction could be correlated with the action of any of the nerves to which vasodilator actions have been attributed. "Vasodilator nerves" have been described for many organs; but in nearly every case their presence has been questioned. In fact the existence of "vasodilator" nerves (in the usual sense in which the term is employed) often has been the subject of discussion (2), (3), (4). Further, some authors believe that certain "vasodilator nerves" should be classed with one group of nerves, others that they should be placed in a different category. For present purposes we may consider the following groups of alleged vasodilators and see what evidence there is that they are involved in the action of acetyl-cholin: (a) Parasympathetic vasodilators, (b) Posterior root vasodilators, (c) Sympathetic vasodilators.

a. Parasympathetic vasodilators. The chorda tympani, stimulation of which causes a dilatation of the vessels of submaxillary gland, and the pelvic nerve, the stimulation of which causes, among other effects, dilatation of the vessels of the penis, have long been considered typical examples of parasympathetic vasodilators. It has been shown in the preceding paper that acetyl-cholin causes vasodilation in the submaxillary gland and in the penis; does it act upon the "nerve-endings"

of the parasympathetic nerves? That this is not the case (at least in the conventional sense) is shown by the different effects of atropine in the two cases: the action of acetyl-cholin is easily prevented by atropine; that of the stimulation of the nerves is not. Thus it was shown in the preceding paper that a small dose of atropine (2 mgm., for example) prevented the marked dilator effect of acetyl-cholin upon the penis; Langley and Anderson (5), Piotrowski (6) (and others cited by Piotrowski) found that atropine (even up to 60 mgm.) did not prevent the vasodilator action of the pelvic nerve.

As regards the chorda tympani: I found, for example, that 0.05 mgm. atropine prevented the vasodilator action of acetyl-cholin upon the submaxillary gland, whereas stimulation of the chorda continued to cause a marked vasodilation after more than 5 mgm. atropine. It has long been a common physiological demonstration that atropine while "paralyzing the secretory fibers" of the chorda tympani does not paralyze its vasodilator fibers (7). It has however been shown (8) (and I have confirmed the results) that atropine does diminish the "vasodilator" action of the chorda tympani and some, Barcroft for example, are inclined to hold that the vasodilation is the result of the secretory processes caused by the stimulation of the nerve. (See (2), (3), (4)). Of course, an interpretation could be placed upon my results with acetyl-cholin, similar to that given by Henderson and Loewi (8) for pilocarpine, namely, that the vasodilation from this was simply the result of increased secretory activity; but similar relations between atropine and acetyl-cholin hold for organs in which an analogous explanation can scarcely be offered. Since, when comparable degrees of vasodilation are caused on the one hand by acetyl-cholin and on the other by stimulation of the chorda tympani, the action of the latter is not perceptibly impaired by an amount of atropine a hundred times greater than that which suffices to prevent the action of the former it seems simpler to suppose that the vasodilation caused by acetyl-cholin is not the result of a stimulation of the chorda "nerve-endings."

It has also been shown that the vasodilators to the tongue are not paralyzed by atropine (6).

b. Posterior root dilators. The most widely distributed and apparently the most powerful vasodilator nerves described are those in the posterior roots which were discovered by Stricker but which have been investigated with especial care by Bayliss (9). Bayliss considers that the vasodilator supply to the limbs, skin of the trunk and probably

of the ears and face, and of the intestine belong to this system. It is in these regions that acetyl-cholin exerts its most pronounced vasodilator action; is this the result of the stimulation of the "endings" of these nerves? Atropine prevents the action of acetyl-cholin upon the vessels in this area, as in all other cases. So far as I can learn the effect of atropine upon the vasodilator action of the posterior roots has not been directly tested. There is, to be sure, some evidence that atropine does not paralyze these nerves. Thus Ostroumoff (10) reported that the vasodilation resulting from weak or slow stimulation of the peripheral end of the sciatic was not prevented by atropine; Pick (11) found that whereas large doses of atropine diminished the action of the vasoconstrictors of this nerve that of the vasodilators was not affected. Bayliss believes that the only sources of vasodilators to the limbs are the posterior root fibers, but others have described sympathetic vasodilators to the limbs, and Gaskell (4) has recently stated that he thinks it impossible to consider that the posterior root fibers are the same as those found in the sciatic by slow stimulation. It seemed desirable therefore to test the effect of atropine upon the result of the direct stimulation of the posterior root fibers. This seemed especially desirable in view of Gaskell's suggestion that the posterior roots affect the metabolism of the skin as the chorda tympani controls the cells of the submaxillary gland and that the vasodilation from the stimulation of the nerves is, in both cases, the indirect result of changes in metabolism (production of "metabolites" which cause vasodilation.) The acceptance of this suggestion would not necessarily lead to the expectation that atropine would prevent the vasodilation caused by the posterior roots for there is no information as to the character of the metabolites supposed, on the above hypothesis, to be produced. But if atropine does not prevent the vasodilator action of the posterior roots whereas it does prevent the action of acetyl-cholin the conclusion would be justified that the vasodilation in the two cases is different.

As a matter of fact, I found that when comparable degrees of vasodilation were caused in a posterior extremity of a dog by stimulation of a posterior lumbar root on the one hand and by acetyl-cholin on the other, the effect of the latter was completely prevented by a small dose of atropine whereas that of the former was not diminished even by large doses (fig. 1, exp. 476).

According to the current conceptions of the posterior root vasodilators (3), (9), (see however, (12)), the nerve fibers of these divide, one

division supplying receptors of the skin, etc., and the other the blood vessels; the former are stated to be readily paralyzed by cocaine. It

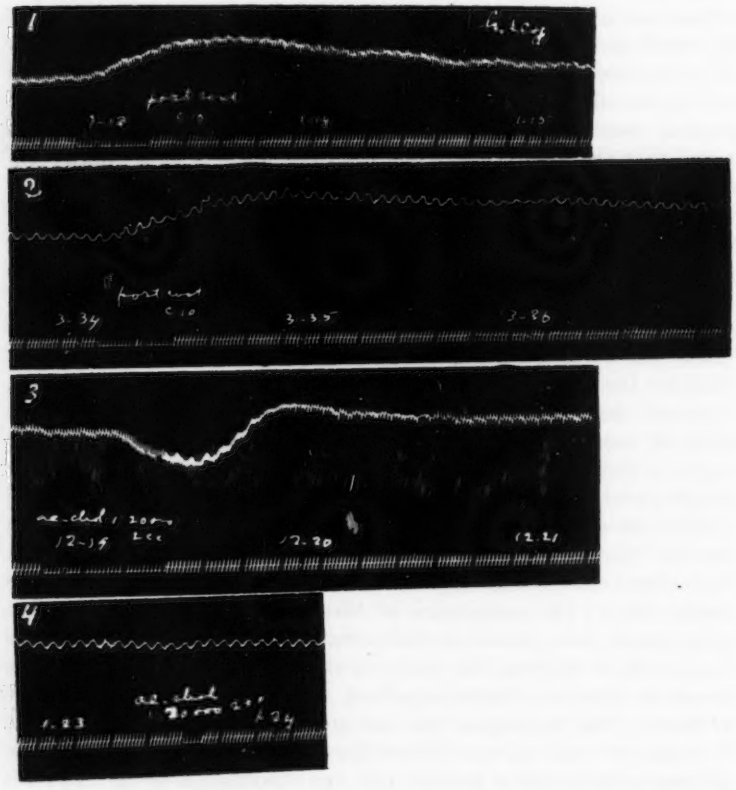

Fig. 1. Experiment 476. Dog, 12.8 K; morphine; ether. Plethysmograph record of hindleg; up = expansion. Time in seconds, 10 sec. and min. (1) *1–13*, stimulation of 7th lumbar posterior root before, and (2) *3–34*, after intravenous injection of 38 mgm. atropine sulphate. (3) *12–19*, 0.1 mgm. acetyl-cholin injected into a paw vein before, and (4), *1–23* after 2 mgm. atropine sulphate. At *12–19* the blood pressure fell from 130 to 71 mm. Hg; at *1–23* there was no change.

apparently has not been determined whether the "vasodilator branches" are paralyzed by cocaine. The acetyl-cholin vasodilator mechanism is not paralyzed by cocaine; thus the dilatation of a cat's leg (plethys-

mograph) caused by injecting acetyl-cholin peripherally into the artery was not in the least diminished by the injection of 30 mgm. of cocaine into the artery; the absolute dilatation was much increased as the acetyl-cholin completely overcame the marked vasoconstrictor action of the cocaine.

c. Sympathetic vasodilators. Several writers have described vasodilator nerves belonging to the sympathetic system; this interpretation has been questioned in nearly every case. Thus Carlson (13) reported the presence in the cervical sympathetic of the cat of vasodilator fibers to the submaxillary gland; he found that these fibers are paralyzed by relatively large amounts of atropine. Some more recent writers (2), (3), have attributed the vasodilation observed by Carlson to the effects of metabolites resulting from the stimulation of the secretory fibers of the sympathetic (a possible explanation considered by Carlson but held by him to be insufficient); the primary action of the atropine would, according to this interpretation, be upon secretory and not upon vasodilator nerves. A similar explanation has been suggested (4) for the vasodilation seen in the bucco-facial region of the dog following stimulation of the cervical sympathetic.[1] Special interest in "sympathetic vasodilators" has been aroused in recent years by the work of Dale, (16), (17), (18). Dale found that after the injection of ergotoxine or of ergot preparations containing ergotoxine into certain animals stimulation of the splanchnic (also after removal of the adrenal), or of the spinal cord, caused a fall of blood pressure; stimulation of the abdominal sympathetic caused a dilatation of the vessels of the foot instead of the usual constriction. Epinephrin (and also nicotine) caused only a fall of blood pressure in these animals and only a dilatation of the intestines and spleen.[2] Dale interpreted these results as probably additional evidence for the existence of sympathetic vasodilator nerves the action of which is usually obscured by that of the sympathetic vasoconstrictor nerves. Similarly the vasodilation, often resulting in a fall of blood pressure, usually obtained (without the previous injection of ergotoxin) in certain animals after the injection of small amounts of epinephrin has often been attributed to a stimula-

[1] This vasodilator action was described by Dastre and Morat (14) and confirmed by Langley and Dickinson (15) and by Dale (16). None of these writers report experiments with atropine.

[2] This effect upon the spleen and also the abolition, by ergot, of the pressor action of epinephrin and of splanchnic stimulation was observed independently by Sollman and Brown (19).

tion of such nerves. The work of Dale and others shows that this vasodilator mechanism is widely distributed in the body;[3] hence it was of interest to see if it was involved in the vasodilator action of acetyl-cholin. There were some reasons for suspecting that the latter would be found not to be the case; for instance, no evidence based upon experiments such as those just described has been found for the presence of sympathetic vasodilators in the rabbit and yet the vasodilator action of acetyl-cholin is very pronounced in this animal.

So far as I am aware Carlson (13) is the only one who has, in recent years, investigated the action of atropine upon so-called sympathetic vasodilators; as was stated above, doubts have been expressed as to the vasodilator functions of the nerve he studied. Ostroumoff argued that the vasodilators in the sciatic which he investigated belonged to the sympathetic system; as was stated above he found that these nerves were not paralyzed by atropine. It has been shown, however, that there are many posterior root vasodilators in the sciatic—perhaps they are the only ones present—these, as was shown above, are not paralyzed by atropine.

If the vasodilation from acetyl-cholin is due, in whole or in part, to an action upon the endings of sympathetic vasodilators or to the same mechanism, whatever it may be, responsible for the fall of blood pressure from epinephrin we should expect to find the action of the latter diminished or prevented by atropine. I have been unable to find any record of experiments in which the effect of atropine upon the vasodilator action of epinephrin after ergotoxine was tested. The only reference which I have found as to the relation of atropine to the depressor action of epinephrin is an incidental remark by Chiari and Fröhlich (24): they found epinephrin to cause a fall of blood pressure in cats poisoned with oxalates and in a normal cat with high blood pressure; they state that the vagi had been cut or atropine administered.

[3] Meltzer and Meltzer (20) reported experiments which suggest that epinephrin may have a central vasodilator action; Pilcher and Sollmann (21) found vascular dilatation to result exceptionally from a central action of epinephrin but believed it to be caused by an increased blood supply relieving an asphyxial stimulation; Hartman and Fraser (22) believe the epinephrin vasodilator reaction to be of central origin. That the epinephrin vasodilation after ergotoxine is not, at least solely, due to a central action is shown by the dilatation observed by Dale in the cat's foot when epinephrin was injected peripherally into the femoral artery after ergotoxine and by the work of Cannon and Lyman (23) who showed that epinephrin may cause a fall of blood pressure, after ergotoxine, when the brain and entire spinal cord have been destroyed.

I have frequently observed, and have no doubt that others have made the same observation, although I do not find it recorded, a fall of blood pressure from epinephrin after atropine had been administered, i.e., in animals which had not received ergotoxine. From a quantitative standpoint such experiments are often unsatisfactory; the results are often obscured by a fact noted incidentally by others (24), (25), and more fully investigated by Cannon and Lyman (23) namely, that the extent of the fall of blood pressure and at times even its occurrence is dependent to a considerable degree upon the level of the blood pressure when the epinephrin is injected. If, as often happens, atropine has caused a fall of blood pressure the depressor action of a given dose of epinephrin may be less or even replaced by a rise. However, a fall of blood pressure from epinephrin after the administration of sufficient atropine to prevent the vasodilator action of acetyl-cholin has been so often observed as to leave no doubt that the fall of blood pressure from epinephrin is of a different character from that after acetyl-cholin; experiments of this kind are shown in tables 3 and 4 (p. 243.)

The same is also shown in the following experiment (which also shows that the depressor action of acetyl-cholin is not altered by ergotoxine).

TABLE 1

Experiment 436. Cat; 1.96 K; pithed from second vertebra upwards; injections into external jugular

TIME	DOSE	BLOOD PRESSURE
11–29	Epinephrin 1: 100,000, 1 cc.	Rose 29 mm. (90 to 119)
36+	Acetyl-cholin 1: 5,000,000, 1 cc.	Fell 16 mm. (80 to 64)
40	Ergotoxine, 5.2 mgm.	Rose 67 mm. (78 to 145)
5	Epinephrin 1: 100,000, 1 cc.	Fell 9 mm. (105 to 96)
9	Acetyl-cholin 1: 5,000,000, 1 cc.	Fell 18 mm. (87 to 69)
51	Epinephrin 1: 10,000, 1 cc.	Fell 15 mm. (83 to 68)
4	Ergotoxine, 3.25 mgm.	0
5	Acetyl-cholin 1: 5,000,000.	Fell 13 mm. (77 to 64)
12–01	Atropine sulphate, 0.5 mgm.	0
5	Acetyl-cholin 1: 100,000, 1 cc.	0
6	Epinephrin 1: 10,000.	Fell 13 mm. (70 to 57)
11	Atropine sulphate, 2.5 mgm.	0
48	Ergotoxine, 3.25 mgm.	Rose 26 mm. (86 to 92)
50	Epinephrin 1: 10,000, 1 cc.	Fell 23 mm. (83 to 60)
12–56 to 1–31	9.6 mgm. atropine sulphate.	
1–34	Epinephrin 1: 10,000, 1 cc.	Fell 28 mm. (92 to 64)
43	Acetyl-cholin 1: 10,000, 1 cc.	Fell 14 mm. (94 to 80)

It will be noted in this experiment that after ergotoxine had been given until the epinephrin effect was reversed and after the injection of 12.6 mgm. of atropine, 0.1 mgm. acetyl-cholin caused a fall of pressure. Ordinarily, i.e., without ergotoxine, such an amount of acetyl-cholin injected after such an amount of atropine caused no fall of blood pressure; frequently it, like larger doses, caused a rise of pressure (nicotine-like action of Dale). Thus it appeared that ergotoxine may have prevented or reversed the pressor action of acetyl-cholin. That ergotoxine does have this action was shown in another experiment: atropine was given until acetyl-cholin (5 mgm.) caused only a rise of pressure (from 78 to 118 mm. Hg.); 6.5 mgm. ergotoxine was injected; the same dose of acetyl-cholin caused the pressure to fall 34 mm. (from 80 to 46). Expressed in the terms of the hypothesis of sympathetic vasodilators and the nicotine-like action of acetyl-cholin this result might be interpreted as showing that acetyl-cholin stimulates ganglion cells of both the sympathetic vasoconstrictors and of the vasodilators; but since the endings of the former had been paralyzed by ergotoxine only the action of the latter appeared, leading to the fall of blood pressure. The injection of nicotine in this experiment was of interest; the first injection (20 mgm.) caused only a pronounced fall of pressure (as was to be expected after ergotoxine: "stimulation of sympathetic vasodilators"): a second injection of nicotine had no effect on the blood pressure ("stage of the paralysis of the sympathetic vasodilators"). But acetyl-cholin caused as great a fall of pressure as before the nicotine, which may be interpreted as indicating that sympathetic vasodilator ganglia are less easily paralyzed by nicotine than are the constrictor ganglion cells. An alternative hypothesis would be that the fall of pressure was due to the acetyl-cholin being able to overcome the peripheral action of the atropine when the possibility of its constrictor action had been eliminated by ergotoxine.

2. THE RELATION OF THE ACETYL-CHOLIN VASODILATOR REACTION TO REFLEX VASOMOTOR CHANGES

It was not possible, as has been shown above, to find a relation between the vasodilator action of acetyl-cholin and any of the groups of nerves to which vasodilator functions have been ascribed: namely, the parasympathetic, the posterior root or the sympathetic nerves. Since, however, the mechanism through which acetyl-cholin exerts its vasodilator action is the most powerful yet found in the body it seemed of interest to determine if there are any indications that the body makes use of it in reflex vasomotor changes and also to compare the areas involved, the degree of activity, etc.

a. Depressor nerve. If the mechanism under discussion is involved in the fall of blood pressure resulting from stimulation of the depressor nerve the effect of the latter should be diminished by atropine. It is, of course, well known that the depressor causes a fall of blood pressure after atropine but no experiments seem to have been reported in

which the possibility was considered that atropine might diminish the effect or possibly prevent one phase of the action. A number of investigators, notably Bayliss (26), have shown that the fall of blood pressure from stimulation of the depressor is due in part to increased activity of vasodilator nerves, in part to an inhibition of vasoconstrictors. Martin and Stiles (27) found two types of reflex fall of blood pressure from stimulation of the central end of the vagus (cat); in one type the threshold is low and the authors believe the fall of blood pressure is due to the excitation of vasodilators; the other type has a high threshold and is believed to represent the inhibition of constrictors. It seemed of interest to determine if atropine might not affect the one type and not the other. My own experiments were performed upon rabbits. The only publication I found bearing upon this subject was by Tschirwinsky (28), who stated that the fall of blood pressure from the depressor was 26.2 per cent before and 29.7 per cent after atropine; he also stated that he sometimes found the depressor to cause a rise of pressure and (if I understand him correctly) that this was facilitated, or increased, by atropine. As regards the latter point: I have occasionally seen the fall of pressure followed by a rise but this seemed to be entirely independent of the administration of atropine. I did not find atropine to have any effect upon the fall of blood pressure resulting from either weak or strong stimulation of the depressor. I do not consider these results to be conclusive evidence that atropine may not modify certain features of the action of the depressor nerves; finer gradations of stimuli might have given different results and plethysmograph experiments might have shown that after atropine the relative extent of dilatation in different organs was changed (see below). But even if such changes were found this would not invalidate the conclusions that the acetyl-cholin dilator mechanism is not involved, to any considerable extent, in the fall of blood pressure from stimulation of the depressor.

Plethysmograph experiments showed that, the blood pressure being high, when equal falls of blood pressure were caused by depressor stimulation and by acetyl-cholin the expansion of the foreleg and the ear was usually greater with the acetyl-cholin. When, as sometimes occurred, the depressor and acetyl-cholin caused a (passive) diminution in the volume of the hindleg that resulting from the former was greater; the fall of blood pressure being the same in both cases. These results suggest that dilatation of the limb and ear vessels are a more important factor in the acetyl-cholin fall of blood pressure than in that

caused by the depressor. Comparisons made upon other organs were not very satisfactory but the following results are of interest: in an experiment in which the blood pressure was very low (39–40 mm.) depressor stimulation and the injection of acetyl-cholin caused about equal falls (9–11 mm.) of blood pressure and a (passive) diminution in the volume of a loop of the intestine; later when the blood pressure was higher (78–82 mm.) acetyl-cholin caused a slight expansion of the intestine and depressor stimulation a slight diminution (the fall of blood pressure being 22 and 24 mm. respectively); a little later, with a blood pressure of about 90 mm. both the depressor and acetyl-cholin cause a marked expansion of the intestine. These results suggest that the dilatation of the intestinal vessels is dependent upon the degree of their tonicity; considered in connection with what follows it seems probable that the depressor dilatation in this area is chiefly due to an inhibition of vasoconstrictors.

There was in one respect, a striking difference between the effects of depressor stimulation and of acetyl-cholin upon the volume of the ear or leg (table 2 and fig. 2). As already stated the acetyl-cholin dilatation was greater than the depressor dilatation although the fall of blood pressure was the same in both cases. Moreover, the dilatation could be obtained repeatedly and without any diminution from acetyl-cholin but the dilatation from depressor stimulation became less and was sometimes replaced by a (passive) diminution of volume, although the effect upon the blood pressure remained the same. A number of factors may be involved (especially in such an experiment as that upon which table 2 is based) in this lessened dilatation of the leg or ear. But the factor apparently most often involved was the repeated stimulation of the depressor itself, and there seems justification for the hypothesis that the dilatation of the limbs and ear from depressor stimulation is chiefly due to a central stimulation of vasodilators and that this mechanism is easily fatigued whereas the acetyl-cholin dilatation is entirely peripheral and is not easily fatigued.

b. Other afferent nerves. Stimulation of afferent nerves other than the depressor will, as is well known, cause a fall of blood pressure, especially under certain conditions. The mechanism of this fall of pressure is not definitely known; that it is different from that involved in the acetyl-cholin reaction is indicated by the fact that it is not prevented by atropine (tables 3 and 4; in both of these experiments the fall was greater after atropine).

TABLE 2

Experiment 457. Rabbit; see figure 2

TIME		BLOOD PRESSURE (FEMORAL)	EAR (PLETHYSMOGRAPH)
12–19	L. depressor stimulated 10 sec., coil 13.5.	Fell 42 mm. (86–44)	Expansion (fig. 2, a)
22	Acetyl-cholin, 0.001 mgm. intravenously..	Fell 36 mm. (86–50)	Expansion (fig. 2, b)
27	L. depressor; 10 sec., coil 13.5.....	Fell 42 mm. (84–42)	Slight expansion (fig. 2, c)
32	L. depressor; 10 sec., coil 13.5.....	Fell 40 mm. (82–42)	0
35½	Acetyl-cholin, 0.001 mgm. (injected more slowly)..	Fell 23 mm. (79–56)	Expansion (fig. 2, d)
37	0.9 per cent NaCl, 1 cc...	0	0
39	Atropine sulphate, 0.5 mgm.....	Fell 8 mm. (83–75)	0
42	L. depressor.....	Fell 40 mm. (81–41)	Slight expansion
48	Acetyl-cholin 0.001 mgm.....	0	0
51	L. depressor.....	Fell 40 mm. (84–44)	Slight expansion
54	Acetyl-cholin, 0.01 mgm.....	Fell 38 mm. (86–48)	Great expansion
58	Chloral hydrate 0.036 gram, Ethyl carbamate, 0.09 gram, (intravenously)		
1–03	L. depressor.....	Fell 35 mm. (91–56)	Great expansion (fig. 2, e)
	Atropine, 2 mgm.....		
1–58	L. depressor.....	Fell 38 mm. (90–52)	Expansion (fig. 2, f)
2–02	Acetyl-cholin, 0.05 mgm.....	Fell 39 mm. (88–49)	Great expansion (fig. 2, g)
07	L. depressor.....	Fell 38 mm. (86–48)	0
	Atropine, 5 mgm.....		
2–39	L. depressor.....	Fell 39 mm. (88–49)	0
42	Acetyl-cholin, 0.05 mgm.....	Fell 20 mm. (88–68)	Great expansion
	Chloral hydrate 0.108 gram, Ethyl carbamate. 0.270 gram, (intravenously)		
3–40	Atropine sulphate 5 mgm.....	Fell 20 mm. (96–76)	Expansion (fig. 2, h)

TABLE 2—Continued

Experiment 457. Rabbit; see figure 2

TIME		BLOOD PRESSURE (FEMORAL)	EAR (PLETHYSMOGRAPH)
3–46	L. depressor.....	Fell 39 mm. (93–54)	0
53	Acetyl-cholin, 0.025 mgm.....	Fell 14 mm. (88–74)	0
4–02	Acetyl-cholin, 0.1 mgm.	Fell 49 mm. (90–41)	Great expansion (like 2–02)
13	Atropine, 10 mgm.....	Fell 42 mm. (93–51)	Great expansion Great contraction
19	Acetyl-cholin, 0.1 mgm.	0	0
30	L. depressor, coil 10....	Fell 22 mm. (72–50)	Contraction

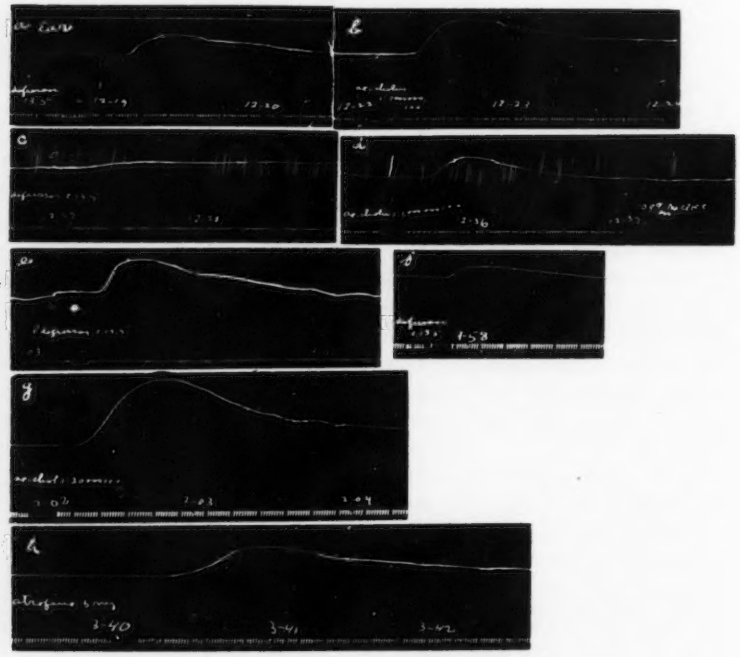

Fig. 2. Experiment 457. Rabbit, 1.84 K; chloral hydrate and ethyl carbamate; vagi cut. Ear in plethysmograph; up = expansion. Time in seconds, 10 sec. and min. See table 2.

TABLE 3

Experiment 473. Cat; 3.78 K; chloral hydrate and ethyl carbamate

TIME	DOSE	BLOOD PRESSURE (FEMORAL)
12–06	Sciatic (Harvard coil; tetanizing current), coil 13	Fell 7 mm. (127–120)*
12–12	Acetyl-cholin, 0.01 mgm. (saphenous vein.)	Fell 50 mm. (114–64)
22	Epinephrin, 0.005 mgm.	Fell 36 mm. (122–86)
25	Atropine sulphate, 1 mgm.	Fell 17 mm. (122–105)
27	Epinephrin, 0.005 mgm.	Fell 29 mm. (11 –88)
31	Acetyl-cholin, 0.01 mgm.	0
45	Epinephrin, 0.01 mgm.	Fell 49 mm. (124–75)
	3.5 mgm. atropine sulphate.	
1–07	Sciatic, coil 13.	Fell 12 mm. (118–106)
12	Epinephrin, 0.01 mgm.	Fell 45 mm. (120–75)
19	Sciatic, coil 13–45	Fell 15 mm. (127–112)†
22	Epinephrin, 0.005 mgm.	Fell 52 mm. (126–74).

*This was the greatest fall of pressure which could be obtained with any strength of stimulus.

† This was the greatest fall of pressure which could be obtained with any strength of stimulus.

TABLE 4

Experiment 460. Cat; chloral hydrate and ethyl carbamate

The sciatic was stimulated a number of times with very weak as well as stronger stimuli; the greatest fall of blood pressure obtainable (coil 13–90) was 4 mm. Cold alcohol was now circulated about the sciatic; the strongest stimuli (which had previously caused only a rise of pressure) now uniformly caused a fall of pressure which was greater than that obtainable with any strength of stimulus before the nerve was cooled; 0.0005 mgm. acetyl-cholin injected intravenously had caused the pressure to fall 27 mm. The nerve was being cooled when the following stimulations were made.

TIME	DOSE	BLOOD PRESSURE (FEMORAL)
12–13	Sciatic, coil 6.	Fell 11 mm. (114–103)
14	Epinephrin, 0.002 mgm.	Fell 10 mm. (110–100)
15	Atropine sulphate, 0.5 mgm.	Fell to 96 mm.
17	Sciatic, coil 6.	Fell 14 mm. (96–82)
19	Epinephrin 0.002 mgm.	Fell 9 mm. (91–82)
26	Sciatic, coil 6.	Fell 10 mm. (98–88)
27	Acetyl-cholin, 0.005 mgm.	0
36	Atropine sulphate, 1 mgm.	
38	Sciatic, coil 6.	Fell 13 mm. (99–86)
40	Epinephrin, 0.005 mgm.	Fell 16 mm. (100–84)
43	Acetyl-cholin, 0.025 mgm.	Fell 4 mm. (103–99)
44	Atropine sulphate, 5 mgm.	
46	Sciatic, coil 6.	Fell 19 mm. (96–77)

The circulation of the alcohol was stopped and the temperature of the nerve allowed to return to normal; stimulation now with any strength of current caused no effect or only a rise of pressure; with the coil at 6, for example, the rise was 41 mm. (93–134); no fall could be obtained with any strength of stimulus.

In the second of the above experiments I made use of a method for intensifying the fall of blood pressure, when ordinary "tetanizing" currents are used, originally described by Howell, Budgett and Leonard (29) and more fully investigated by me (30) viz., stimulating the nerve while it was being cooled. So far as I am aware Vincent and Cameron (31) are the only investigators who have repeated these experiments; they state that they obtained the same results (although their interpretation of the results was different from that which I thought could be placed upon them.) Others, without however repeating the experiments, have expressed the opinion that the effect of the cooling is simply equivalent to that of employing a weaker stimulus. Thus Martin and Lacy (32) suggested that the procedures I employed (among them cooling the nerve) resulted in a mere impairment of conductivity:

"Thus strong stimuli were converted into weak ones, and what Hunt observed was the usual effect on blood pressure of threshold stimulation." Ranson and Billingsley (33) stated: "Cooling a nerve would decrease the strength of the impulses passing over it so that the net result would be the same as that of weak stimulation." I thought that I had met objections of this character (which, moreover, at least when expressed in this way, are difficult to reconcile with the current views of the "all or nothing" principle of the nerve impulse) when I stated that I had found that "a much greater fall of pressure can be obtained when the nerve is cooled and stimulated, than can be obtained by the use of a weak stimulus alone"—a fact again illustrated in the experiment cited above. I also found that stimulation of the central end of a regenerating nerve caused a fall of pressure; Vincent and Cameron found the same. These results have been interpreted in the same way as the cooling experiments; it has been said "the conductivity of a regenerating nerve is less than that of a normal one and only weak impulses could be made to reach the cord by way of such a nerve." I had pointed out, however, that a greater fall of pressure could be obtained from a regenerating nerve than could be obtained with any strength of stimulus from the corresponding normal nerve (unless the latter were cooled).

Ranson and Billingsley expressed surprise that Vincent and Cameron should have not always found it possible to satisfy themselves as to the different effects of weak and strong currents; they themselves considered a rise of pressure from weak stimulation atypical. Martin and Lacy also speak of the constancy with which they obtained a fall of pressure with weak stimuli. My experience on the whole has been similar to that of Vincent and Cameron; it has been somewhat exceptional for me to obtain marked falls of blood pressure with weak stimuli. The experiments of Gruber (34) offer a satisfactory explanation of these differences. Gruber found that the rate of stimulation has fully as great an influence upon the result as the strength of the stimulus. I had tried different rates in my original experiments but for some reason failed to find a difference. Martin and Lacy seem also to have obtained negative results in such experiments for Stiles and Martin (35) state: "Martin and Lacy have found that the interruption of the primary current can be made to take place at widely varying rates without affecting the extent of the vasomoter change resulting from the stimulation of a single afferent path." I find it very easy to confirm Gruber's statement as to the difference between the effects of different rates of stimulation; this is a more convenient method of obtaining a depressor reaction than the

method of cooling. Martin and Lacy do not state the rate of stimulation they employed; they simply say that an apparatus was used by which the primary circuit was broken 2 to 8 times a second and that for more rapid rates an apparatus was used by which the circuit could be broken from 4 to 60 times a second. In later papers on this general subject Martin and his coworkers speak of using 8 to 15 (36), 5 to 16 (37) and 8 to 12 (38) interruptions per second. Ranson and Billingsley do not state definitely the rate of stimulation they employed but refer to an earlier paper (39) in which it was stated that the rate "was fairly slow, about 25 per second." Gruber used rates of from 4 to 20 per second. On the other hand Howell, Budgett and Leonard, Vincent and Cameron and I employed the "tetanizing" current; in the coil I used, the rate was about 80 per second. The group of investigators who used slower rates found a fall of pressure to be the usual result of the stimulation of an afferent nerve with weak stimuli; those of us who used more rapid rates often failed to observe more than slight falls of pressure. This difference in the method of experimenting may also explain a difference in the results which have been reported for certain nerves: thus I, using the tetanizing current, found in a considerable number of experiments a fall of pressure from the saphenous nerve in the dog and rabbit, but stated that it "rarely occurred" from stimulation of this nerve in the cat; Langley (40) found the same, although he states that his experiments upon cats were not numerous. Other workers using slower rates of stimulation have easily obtained a fall of pressure from the cat's saphenous. I have recently obtained the same.

I have recently made, incidentally, some observations upon the effects of cooling a nerve (sciatic, cat) and stimulating it with the "tetanizing" current (about 80 interruptions per second) and with a slow rate of stimulation (6 per second). Before cooling only a very slight fall of pressure (4 or 5 mm.) could be obtained whatever was the position of the secondary coil of the inductorium arranged for the tetanizing current. The strength of the latter was increased until a rise of 15 mm. was obtained; a fall of 14 mm. was obtained with the slow stimulation. Cold alcohol was circulated about the nerve in the manner described in my original paper and the nerve stimulated alternately with the slow and with the rapid rate, the secondary coils remaining the same in each case. As the nerve was cooled the rise of pressure from the tetanizing current became less, then there was no change and finally only a fall (15 mm.) was obtained. The fall of pressure from the slow stimulation remained the same. If, as has been suggested, the change from the rise to the fall of pressure in the former case was simply the result of a weakening of the stimulus through cooling it is difficult to see why the depressor reaction from the slow stimulation was not lessened. In this experiment the fall of pressure from the slow rate (before and after cooling), which was the maximum effect which could be obtained, was the same as the maximum fall obtained by cooling the nerve and stimulating it with the tetanizing current.

I thought (30) that the fall of blood pressure which results under certain conditions from the stimulation of afferent nerves (it being assumed that the condition of the center remained unchanged) could, on the whole, be more satisfactorily explained on the hypothesis that there are present in mixed nerve trunks depressor, or reflex vasodilator, nerve fibers rather than on the hypothesis

that stimulation of the same nerve fibers may at times lead to a fall, at other times to a rise of blood pressure. The chief objection to this hypothesis has been based upon the quantitative studies of Martin and Lacy on vasomotor reflexes from threshold stimulation; Ranson and Billingsley (33) found this work to be the "most serious difficulty" in the way of such a hypothesis. As they say: "according to them (Martin and Lacy) the threshold for the depressor reflex is 8.7 units as compared with . . . . 280 units for the pressor reflex." "It is difficult to conceive of two afferent fibers so differently constituted that the stimulation threshold of one should be 30 times greater than that of the other." This particular difficulty seems to have been removed by Gruber's work: Gruber obtained pressor responses with only 5.8 units if the rate of stimulation were increased to but 20 per second; on the other hand, depressor responses were obtained with 383 units (about 70 times stronger) if the rate of stimulation was reduced to 4 per second. With a still slower rate of stimulation a depressor response was obtained with 6643 units, which was 390 times stronger than the current necessary to produce a rise of blood pressure when interrupted 20 times per second. Gruber concludes that the threshold for both pressor and depressor responses may be the same depending upon the rate.

Little positive evidence has been adduced by those who believe that only one set of afferent fibers is involved in both pressor and depressor reactions. Martin and Lacy seemed to think that their studies on the reflex vasomotor thresholds and the assumption that cooling a nerve, etc., was equivalent to merely weakening the stimulus was sufficient to make this hypothesis at least equally possible; in later publications, however, Martin and his coworkers have had occasion to make use of the other conception.

Ranson and Billingsley adopt a hypothesis which is essentially the same as one which I considered in 1895 and thought inadequate; I stated it as follows: "We might suppose one nerve fiber to be connected with both a dilator and a constrictor center; and perhaps we might further imagine that a weak stimulus could reach or excite one center more readily than the other, owing to a difference in irritability." I endeavored, unsuccessfully, to trace the paths of the pressor and depressor impulses in the spinal cord but Ranson and Billingsley have made the very important discovery of tracts in the spinal cord by which "a weak stimulus could reach or excite one center more readily than the other." They found that the "depressor path" is through a tract of long fibers with few relays whereas the pressor path is through a series of short relays. Accepting the views of Martin on the relation between strength of stimulus and the vasomotor response they believed that a weak stimulus could readily reach the centers causing a vasodilation through the former, whereas only stronger impulses could pass through the pressor path to the centers causing a vasoconstriction. This attractive hypothesis, however, has lost its chief support through the work of Gruber although doubtless it could be employed equally well to explain the effects of different rates of stimulation. The possibility that the effect of cooling a nerve is in reality the same as reducing the number of stimuli might also be suggested as a working hypothesis.

On the other hand the fact that pressor and depressor impulses follow different paths in the spinal cord is not in the least inconsistent with the hypothesis that the impulses reach the spinal cord through different nerve fibers. The ana-

tomical relations of the latter are not sufficiently known to make theorizing, beyond the making of working hypotheses, profitable. But there are certain facts and views in this connection which are worthy of consideration. It seems to be generally believed that the posterior root vasodilators are myelinated nerve fibers; the work of Bayliss makes it probable that the stimulation of the central end of these fibers causes at least local reflex vasodilation and my own work indicated that the vasodilation accompanying the fall of pressure from stimulation of afferent nerves occurred in regions supplied by the posterior root dilators. The afferent fibers causing a reflex rise of blood pressure were found by Ranson and Billingsley (41) to be unmyelinated fibers; these authors state that they believe the afferent fibers involved in the depressor reflex to belong to this group although they do not seem to have investigated this point. Should these conceptions prove correct, viz., that the afferent depressor fibers are myelinated and the afferent pressor fibers are unmyelinated they might afford an explanation of the effects of different kinds of stimuli. It is interesting that the methods of stimulation which have proved of value in demonstrating the presence of vasodilator nerves in nerve trunks containing vasoconstrictors, viz., weak stimuli, a slow rate of stimulation, stimulation of a cooled or of a regenerating nerve are the same as those by which a reflex fall of blood pressure may most easily be obtained. To what extent these differences are due to differences in the response of the end or central organs and to what extent they may be due to differences in the nerve fibers themselves has not been satisfactorily determined.

3. ANTAGONISTIC AND OTHER ACTIONS OF DRUGS IN RELATION TO THE VASODILATOR ACTION OF ACETYL-CHOLIN

I have repeatedly called attention to the antagonistic action of atropine to the vasodilator action of acetyl-cholin; in fact this is one of the most characteristic pharmacological reactions of acetyl-cholin yet discovered. Some comparisons were reported in a previous publication (42) between the effects of atropine upon the cardio-inhibitory nerves and its action in diminishing or abolishing the fall of blood pressure from acetyl-cholin. It was found that other members of the "atropine" series (scopolamine, hyoscyamine, homatropine and "eumydrin," or atropinemethyl nitrate) had a similar action but there were marked quantitative differences; in general there was a parallelism between their power to paralyze the cardio-inhibitory nerves and to prevent the vasodilator action of acetyl-cholin.

Recently the action of atropine sulphuric acid ester ("Atrinal") was tested. This was far less active than atropine sulphate; its paralyzing action upon the cardio-inhibitory nerves was also relatively slight, as had already been found by Trendelenburg (43). Thus 1 mgm. of this compound (which when injected intravenously into a

cat caused a fall of blood pressure with a dilatation of the leg and a probably passive contraction of the nasal mucosa) reduced the absolute fall of blood pressure caused by 0.002 mgm. acetyl-cholin by 41 per cent; the percentile fall of pressure was reduced from 35.8 to 30 and the dilating action of acetyl-cholin upon the leg was diminished. Within six minutes, however, the above dose of acetyl-cholin was almost as active as at first; 0.5 mgm. of atropine sulphate completely abolished the effect of 0.002 mgm. acetyl-cholin and greatly reduced that of 0.1 mgm.

Pilocarpine in doses causing no lasting fall of blood pressure, but which usually caused a slowing of the heart, caused a diminution of the acetyl-cholin fall of pressure and less expansion of the rabbit ear; there was a similar diminution in the acetyl-cholin effect in the cat and dog without a slowing of the heart. Unfortunately no injections of acetyl-cholin were made immediately after, or simultaneously with, the pilocarpine injection; it is very probable, from experiments which will be described later, that there would have been a summation of the effects of the two. The depression of the vasodilator action of acetyl-cholin by pilocarpine is doubtless similar to the depression of the vagus effects of "synthetic muscarine" by pilocarpine described by Gaisböck (44); in fact Gaisböck's protocols show effects upon the blood pressure similar to those I obtained with pilocarpine and acetyl-cholin but he attributed the effects to a cardiac action.

Physostigmine which is often classed, pharmacologically, with pilocarpine has an effect the opposite to that of the latter in relation to acetyl-cholin. Physostigmine was shown in a previous communication (45) to have a sensitizing effect on the cardio-inhibitory action of one of the homocholins and also, apparently, on the pupil-constricting action of the latter; physostigmine has been found to have a similar effect on all of the vascular actions of acetyl-cholin: it increases the fall of blood pressure and the slowing of the heart before a large dose of atropine and the rise of blood pressure after atropine. Some of these actions are illustrated in the following experiment: (Table 5).

A large number of other compounds were tested in the hope that some of them might throw more light upon the action of acetyl-cholin. Thus certain substances which have, or which have been supposed to have, some of the actions of atropine were tested; also a number of substances which have been stated to increase, or diminish, the sensitiveness of the parasympathetic nervous system (upon which most of the effects of acetyl-cholin are exerted). It has been claimed that

whereas atropine paralyzes chronotropic and inotropic cardio-inhibitory functions equally, other substances depress especially the inotropic functions. It seemed of especial interest to test some of these, for the negative inotropic action of acetyl-cholin upon the heart

TABLE 5

Experiment 479. Dog; morphine; ether; vagi cut

TIME		BLOOD PRESSURE	HEART RATE IN 10 SECONDS
2–19	0.05 mgm. acetyl-cholin..	Fell 55 mm. (103–48)	Slowed from 33 to 21
25	0.02 mgm. acetyl-cholin..	Fell 49 mm. (104–55)	0
28	2.0 mgm. atropine sulphate		
41	0.02 mgm. acetyl-cholin..	0	0
43	1.0 mgm. physostigmine salicylate.....	Rose 11 mm. (104–115)	0
47	0.02 mgm. acetyl-cholin..	Fell 17 mm. (113–96)	0

Before the injection of atropine acetyl-cholin caused an expansion of a foreleg (plethysmograph); this was prevented by the atropine but occurred again after the physostigmine.

1 mgm. atropine sulphate and curare were now injected.

3–38	0.1 mgm. acetyl-cholin...	0	0
43	0.3 mgm. physostigmine salicylate.....	0	0
44	0.1 mgm. acetyl-cholin...	Rose 14 mm. (67–81)	0
47	0.4 mgm. physostigmine salicylate.....	0	0
48	0.1 mgm. acetyl-cholin...	Rose 28 mm. (73–101)	Increased from 25 to 28
56	1.8 mgm. physostigmine salicylate.....	0	0
57	0.1 mgm. acetyl-cholin...	Rose 40 mm. (86–126)	0
4–08	6 mgm. physostigmine salicylate.....	0	0
13	5 mgm. acetyl-cholin.....	Fell	Stopped for 40 sec.
17	20 mgm. atropine sulphate.....		
22	5 mgm. acetyl-cholin	Rose 59 mm. (41–100)	Increased from 17 to 25

The acetyl-cholin at 3–38 had no effect on the volume of the leg; the subsequent injections (i.e., after the physostigmine) caused an expansion except that at 4–13 there was a contraction (doubtless passive) and at 4–22 a marked contraction (active because the blood pressure rose).

(marked upon the auricle, less distinct upon the ventricle) is more pronounced than the negative chronotropic action, which appears only after large doses; the action of acetyl-cholin upon the blood vessels may be analogous to this negative inotropic action upon the heart.

Since the dilatation of the blood vessels of glands by acetyl-cholin might be interpreted as an indirect effect of the stimulation of secretion (production of "metabolites") a number of compounds supposed to have effects upon certain phases of metabolism were also tested. Some others were tried on the basis of hypotheses which need not be discussed; others were tested incidentally to other studies. It is quite probable that some of the results would not have been reported negative had larger doses been employed or if the experiments had been performed in a different manner; I was looking for striking, undoubted effects like that of atropine.

Curare which has been stated by some to paralyze vasodilator nerves (denied by others (11)), usually diminished the absolute and percentile fall of pressure from acetyl-cholin when it had itself caused a fall of pressure; when curare caused a rise of pressure, as sometimes occurs, especially in rabbits, the action of acetyl-cholin was slightly increased.

Agaric acid the action of which upon the sweat glands has long been supposed to be analogous to that of atropine (although recently an entirely different interpretation has been given (46) and which is also said to have an antagonistic action to the negative inotropic effect of pilocarpine upon the frog heart (47) had, in a dose of 0.1 gram (cat), no effect upon the acetyl-cholin, or pilocarpine, fall of blood pressure. "*Euphthalmin*," a compound closely related to betaeucaine, which dilates the pupil, presumably by an atropine-like action, seemed to diminish the effect of acetyl-cholin slightly. *Betaeucaine* itself seemed to slightly diminish the fall from acetyl-cholin; it quickly abolished the slowing of the heart from vagus stimulation and for a brief period diminished the slowing from large doses of acetyl-cholin.

"*Novocaine*," which caused a fall of blood pressure and a slowing of the heart, diminished the percentile fall from acetyl-cholin. "*Alypin*" in a dose causing a considerable fall of blood pressure diminished the effect of acetyl-cholin but the latter returned as the blood pressure rose towards normal. *Cocaine* (cat; 36 mgm.) diminished the absolute but not the percentile fall of pressure from acetyl-cholin.

Ergotoxine in doses which reversed the effect of epinephrin upon the blood pressure did not distinctly reduce the effect of acetyl-cholin (table 1). *Histamin* (B-iminazolylethylamin) increased the fall of blood pressure from acetyl-cholin in the cat; there seemed to be a simple summation of the effects of the two. During the low blood pressure following an injection of *peptone* or *hirudin* both the absolute and percentile fall of pressure from acetyl-cholin were diminished; as the blood pressure returned to normal acetyl-cholin had its full effect. Peptone caused an expansion of the liver; acetyl-cholin usually a diminution at least at

first. The previous injection of extract of *corpus luteum* had no effect upon the acetyl-cholin fall of blood pressure; injected together there was a summation of their depressor effects. The action of corpus luteum extract was diminished but not prevented by atropine.

Nicotine. In my first experiments with acetyl-cholin I found that its depressor action was not prevented by nicotine; the percentile fall of pressure might be the same whether the blood pressure was 160 mm. (under the influence of nicotine) or only 40 mm. Dale showed that after atropine large doses of acetyl-cholin caused a rise of pressure; I had observed the same but had failed to interpret it correctly. Dale attributed this rise of pressure to a nicotine-like action of large doses of acetyl-cholin; it is prevented by nicotine. I have found the same but have also observed that sometimes the effect of nicotine is to convert the pressor action of acetyl-cholin (after a small amount of atropine) into a depressor action; after a further injection of atropine the depressor effect disappeared. The pressor action (after atropine, but before nicotine) was probably due to the preponderance of the stimulation of the ganglion cells over that of the peripheral action which had been reduced but not abolished by incomplete atropinization; the depressor action to the preponderance of the latter after the ganglion cells had been paralyzed by nicotine; this depressor action was in turn prevented by sufficient atropine. But the depressor action of acetyl-cholin (after nicotine) sometimes persisted after much atropine had been injected; this can perhaps be best explained on the hypothesis advanced above: nicotine paralyzes sympathetic constrictors more readily than sympathetic dilators and the vasodilation from acetyl-cholin after nicotine and atropine may be due to a stimulation of sympathetic vasodilators. Nicotine also increased the tendency of acetyl-cholin to cause a slowing of the heart. *Emetine* had no effect on the action of acetyl-cholin. The relation of *epinephrin* to the acetyl-cholin fall of blood pressure was discussed in an earlier publication; fig. 12, (p. 214 of preceding paper) shows the balancing action of the two upon the rabbit ear.

The alkaloids of *Zygadenus*, which I (48) showed in 1902 to belong to the veratrine group (the toxic principle of these plants had previously been supposed by some to be colchicine, by others to be a sapotoxin) had no definite effect upon the action of acetyl-cholin; when they caused an extreme fall of blood pressure the percentile fall from acetyl-cholin was usually lessened but as the blood pressure returned, acetyl-cholin had its full effect. Similar effects were obtained with a commercial specimen of *"veratrine."* The experiments with *Yohimbine* were inconclusive; there may have been a slight diminution of the acetyl-cholin effect. Acetyl-cholin was active after large doses of *brucine* and also after *picrotoxin*.

Sodium oxalate which is stated (24) to increase, through deprivation of calcium, the excitability of the autonomic nervous system to pilocarpine and epinephrin, had, in small doses, no effect upon the acetyl-cholin fall of blood pressure; in one experiment (cat) 190 mgm. of sodium oxalate increased both the absolute and percentile fall of pressure from acetyl-cholin. *Magnesium sulphate* diminished the absolute fall from acetyl-cholin and, when it had caused a pronounced fall of blood pressure, also the percentile fall; calcium chloride, after magnesium sulphate, increased first the absolute fall and then restored the percentile fall. During the rise of blood pressure from *barium chloride*, acetyl-cholin sometimes caused a smaller fall of blood pressure; usually, however, the absolute fall was

the same although the percentile fall was less. If, however, the blood pressure had been very low both the absolute and percentile fall from acetyl-cholin was increased after barium chloride. Both the absolute and percentile fall from acetyl-cholin was increased during the rise from *tetra-hydro-B-naphthylamin*. In one experiment (dog) acetyl-cholin seemed much less active after *sodium nitrite*, although the blood pressure was still moderately high.

The fall of blood pressure from acetyl-cholin injected two or three minutes after the injection of *hydrocyanic acid* was markedly diminished (from 40 to 9 per cent for example); there was also a greater tendency of the acetyl-cholin to cause a slowing of the heart (vagi cut). This effect was of very brief duration. The rise of pressure from epinephrin was similarly reduced. *Hydrochloric acid* in an amount sufficient to cause slowing and irregularity of the heart had no effect upon the action of acetyl-cholin. The fall of blood pressure from acetyl-cholin after the intravenous injection of *chloral hydrate* was sometimes slightly increased, sometimes slightly diminished; a constant effect of the chloral hydrate was to increase the tendency of the acetyl-cholin to cause slowing and often irregularity of the heart, a result similar to that obtained by Loewi (49) with "synthetic muscarine" and pilocarpine. *Chlorbutanol* diminished both the absolute and percentile fall of blood pressure in one experiment. A large dose of *sodium diethyl-barbiturate* diminished the effect of acetyl-cholin; *ethyl carbamate* (0.4 gram intravenously; dog 8, 6 K) had no effect; *morphine* diminished the absolute and percentile fall for a short time.

Camphor injected with acetyl-cholin diminished slightly the fall of blood pressure from the latter; no definite results were obtained with *caffeine*. When acetyl-cholin was injected during the rise of blood pressure from *strophanthin* both the absolute and percentile fall was diminished; later both were increased.

After extensive *hemorrhage*, leading to a low blood pressure, the absolute fall from acetyl-cholin was diminished but the percentile fall was increased; the percentile fall from stimulation of the depressor was increased whereas epinephrin caused little or no rise of blood pressure immediately after the hemorrhage. (These results suggest that at some period after hemorrhage those blood vessels which usually respond to acetyl-cholin and depressor stimulation with dilatation and to epinephrin with constriction are in a condition of strong tonic contraction). *Asphyxia* was very effective in causing a rise of blood pressure when this had been lowered to a considerable extent by the application of acetyl-cholin to a lung; stimulation of the *splanchnic* caused a greater rise (both absolute and percentile) under these circumstances but the level reached was not so high. On the other hand the fall of blood pressure from acetyl-cholin was increased when injected during stimulation of the splanchnic or of the spinal cord, but the level reached was not so low, i.e., there was an algebraic summation of the two effects; this also occurred when a sensory nerve was stimulated (for a pressor response) and acetyl-cholin was injected.

Removal of the *adrenal glands* had no immediate effect on the fall of blood pressure from acetyl-cholin. In one experiment (cat; paraldehyde) the adrenals were removed and the same amount of acetyl-cholin injected every fifteen minutes for four and a half hours; during this time the blood pressure fell from 123 to 32 mm.; the absolute fall from the acetyl-cholin steadily declined but the percentile fall remained about the same. It has been supposed by some (with very

little basis; cf. (50)) that after the removal of the adrenals there is an accumulation of cholin in the blood. The *thyroids* were removed in another experiment (cat; paraldehyde) and the reaction to acetyl-cholin tested every twenty minutes for seven and a half hours; the blood pressure fell from 137 to 59 mm.; the absolute fall from acetyl-cholin diminished but the percentile fall remained about the same until near the end when it increased slightly. In another experiment a standard solution of acetyl-cholin was injected every fifteen minutes; between the injections both cervical sympathetics were stimulated, the pupils being kept in a condition of maximal dilatation; the percentile fall of pressure from acetyl-cholin was the same after more than two hours. Twenty-six cubic centimeters of 1: 200,000 solution of epinephrin were now slowly (in sixteen minutes) injected intravenously; when the blood pressure had returned to normal acetyl-cholin was again injected; both the absolute and percentile fall of pressure were very slightly less than before the epinephrin. It has been reported (51) that stimulation of the cervical sympathetic and the injection of epinephrin increases the secretion of the thyroid. It would seem from these experiments that there is no special relation between the condition of the thyroid and the acetyl-cholin fall of blood pressure. The cat in which the cervical sympathetics were stimulated had been given, per os, sodium iodide for several days preceding the experiment; Seidell and I (52) had found in 1908 that inorganic iodides given per os increased within twenty-four hours the amount of physiologically active iodine in the thyroid. (Marine and Rogoff (53) have found, when the iodide was injected into the circulation, a definite increase in the pharmacological activity of the thyroid after the eighth hour; this was well marked by the twentieth hour).[4]

4. DO OTHER VASODILATOR DRUGS ACT UPON THE SAME MECHANISM AS DOES ACETYL-CHOLIN?

The chief characteristic of the acetyl-cholin reaction is that it is readily prevented by atropine; the best available criterion as to whether other drugs act through the same mechanism is to test their activity before and after the administration of atropine. A large number of drugs have been tested in this manner; only a few need mention here.

[4] I may add in this connection that I found in certain experiments on cats in which the thyroids had been removed and the brain and spinal cord pithed to the mid-thorax a progressive increase in the rise of blood pressure from the injection of a small dose of epinephrin; after two hours the increase reached in one case 400 per cent. Levy (51) had found a similar increase in certain experiments; he attributed it to a discharge of thyroid secretion due to a discharge of epinephrin as a result of struggling under ether. But this explanation would hardly hold in the case of the experiment cited above for the thyroids had been removed twenty-four hours previously. I thought the increased response to epinephrin might be due to a gradual loss of irritability of vasodilator mechanisms; if this is the case the latter are not analogous to the acetyl-cholin reaction for this does not seem to diminish during an experiment.

Vasodilator action of pilocarpine. Few studies seem to have been reported on the action of pilocarpine upon the blood vessels. Kobert (54) stated that it constricts the vessels of the perfused leg and kidney. Brodie and Dixon (55) found it to constrict the blood vessels of the perfused limb and intestine; they stated that its action is identical with that of epinephrin except that larger amounts are necessary; after apocodeine or curare they found pilocarpine to cause no constriction but it may now cause a dilatation. Dixon (56) states that the constriction is prevented by atropine. Brodie and Dixon, and Baehr and Pick (57), found pilocarpine to dilate the vessels of the lung; Berezin (58) reported a constriction. Dixon and Halliburton (59) found a slight dilatation of the cerebral vessels. Fröhlich and Pick (60) found pilocarpine to dilate the blood vessels in frogs. Henderson and Loewi (8) carefully investigated the vasodilation in the submaxillary gland following pilocarpine and concluded that it is probably the result of the vasodilator action of products of secretion; Sollmann (61) suggests that the hyperemia of the skin often seen after pilocarpine may possibly be due to the increased activity of the sweat glands.

Thus no one seems to have reported a direct vasodilator action of pilocarpine upon the systemic blood vessels of mammals; my own experiments, on the contrary, point to such an action and also show that this, like the acetyl-cholin vasodilation, is abolished by atropine. Whether the difference between my results and those of others was due to differences in dose was not determined; it is not improbable that large doses of pilocarpine, like very large doses of acetyl-cholin, have a vasoconstrictor action. Under some conditions pilocarpine causes a marked rise of blood pressure. The vasodilator action of pilocarpine is shown in a number of figures in the preceding paper: figure 4 (p. 205) shows the expansion of the leg of a dog; figure 3 (p. 204) that of the leg of a cat after pilocarpine; similar results were obtained with the rabbit. Figure 13 (p. 214) shows the expansion of a rabbit's ear after pilocarpine; this figure also shows the contraction of the ear after epinephrin (which has been said to have the same effect upon peripheral blood vessels as pilocarpine). The effect of pilocarpine upon the outflow of the perfused rabbit ear is shown in figure 10 (p. 212); the effect on the outflow from the submaxillary gland is shown in figure 15 (p. 216). Pilocarpine caused a slight contraction (passive?) followed by an expansion of the dog's penis (one experiment). In all of the above experiments pilocarpine caused a fall of blood pressure with little or no slowing of the heart; both the fall of pressure and the

effects upon the blood vessels (with the exception of the perfused rabbit ear) were readily prevented by atropine; in none of the experiments had curare (which has been stated to prevent the "vasoconstrictor" action of pilocarpine) been given.

In a number of cases comparisons were made between the effects upon the peripheral blood vessels of pilocarpine and acetyl-cholin in doses causing approximately the same fall of blood pressure; the expansion of the rabbit ear (fig. 13, p. 214) and usually that of the leg (fig. 3, p. 204) was slightly less after pilocarpine than after acetyl-cholin; that of the dog's penis (one experiment) was greater; pilocarpine caused a greater increase in the outflow of the submaxillary gland than did acetyl-cholin; it had practically no effect on the outflow from a muscle when acetyl-cholin caused a slight increase; it was less active in causing an increased outflow from a cutaneous vein of the upper part of the leg of a cat (paw removed) than was acetyl-cholin. The last experiment was performed to determine whether the expansion of the intact limb could be attributed entirely to the effect of pilocarpine upon sweat glands—production of "metabolites;" the fact that pilocarpine dilated the skin vessels independently of those of the paw as well as the dilatation of the vessels of the rabbit ear and of the dog's penis indicate that the dilator action is not due entirely to its stimulation of recognized secretory processes. On the other hand the greater effect of pilocarpine as compared with that of acetyl-cholin upon the outflow from the submaxillary gland (fig. 15, p. 216) suggests that here stimulation of secretion may be an important factor in the vasodilation; pilocarpine is a much more powerful stimulant of secretion than is acetyl-cholin.

As to other effects of pilocarpine upon the blood pressure; in dogs, before atropine, the fall of pressure was frequently followed by a rise; after atropine there was often a rise of pressure in cats, dogs and rabbits but with the doses employed (which seldom exceeded 10 mgm.) the rise was not very great except in one case (dog) in which 10 mgm. caused the pressure to rise 57 mm. (from 75 to 132). In one experiment (cat) the rise of pressure was less after removal of the adrenals. In most of these experiments doses of pilocarpine having little or no effect upon the heart rate were employed. How little effect, however, great changes in the heart rate may have upon the fall of blood pressure from the same dose of pilocarpine was strikingly shown in an experiment on a dog (519): the vagi were in a condition of strong activity from which the heart could be freed by cooling the vagi; the

slow rate could be restored by stimulating the vagi peripherally to the cooled part: the fall of pressure from a given dose of pilocarpine was practically the same, 60 to 67 mm. (from 130 or 134 to 63 or 70) whether the heart rate remained the same (18 in 10 seconds) or whether it, following the pilocarpine injection, increased (from 21 to 39) or whether it was slowed slightly (30 to 28). The percentile fall was the same before and after clamping the auriculo-ventricular bundle in one experiment (dog); the same was true for acetyl-cholin; in both cases the fall was prevented by atropine.

Colchicine. Dixon and Malden (62) stated that after colchicine "the blood pressure falls as the result of a very slight cardiac inhibition, and the effect is not obtained on the atropinized animal." They publish a tracing from a dog showing a fall of blood pressure and a contraction followed by a less marked expansion of the hind leg of a dog. This curve is very like the curve I often obtained with acetyl-cholin when the hindleg of an animal was placed in a plethysmograph (see fig. 1, p. 234). I believe the diminution of volume to be passive and due chiefly to a vasodilator reaction occurring in other vascular areas. For when the foreleg of a cat was placed in a plethysmograph and colchicine injected intravenously there was, with the fall of blood pressure, an expansion of the leg; the curves obtained were practically identical with those obtained from acetyl-cholin and pilocarpine (see figs. 2 to 7 and 19 of preceding paper) and need not be reproduced here. Comparatively large doses (5 to 10 mgm.) were necessary to cause a marked expansion; smaller doses (1 mgm., for example) caused some expansion and increased the size of the pulse waves, another indication of vasodilation. A small dose (1 mgm.) injected peripherally into the femoral artery caused a marked expansion of the hindleg with no change in the blood pressure. The fall of blood pressure and the expansion of the leg were prevented by a small dose of atropine. Thus it seems that colchicine has a vasodilator action similar to that of acetyl-cholin and pilocarpine.

The first effect of a small dose of *physostigmine* was a slight fall of blood pressure and an expansion of the foreleg; this was followed by a rise of blood pressure and a contraction of the leg. These effects were prevented by atropine. Physostigmine thus seems to have a vasodilator action of the acetyl-cholin type but this is generally obscured by other more important actions.

Histamin. Histamin (B-imazolylethylamin) causes a fall of blood pressure and vasodilation in some animals the exact cause of which is

in some doubt (63), (64), (65). I do not find experiments recorded in which this drug was given after atropine and it seemed possible that the vasodilation was similar to that caused by acetyl-cholin and pilocarpine. I found, however, in experiments on cats, that the fall of pressure from histamin was not distinctly affected by atropine; frequently the fall of pressure was the same after doses of atropine which abolished the fall of pressure from acetyl-cholin; sometimes the absolute fall was less but the percentile fall remained the same. With a lowered blood pressure, after atropine, the fall might be less but the level reached remain about the same; if the blood pressure was raised by barium chloride the histamin fall of pressure was increased. As already noted I found, as had Barger and Dale (64) a dilatation of the cat's leg after histamin; this was not as great as that caused by an amount of acetyl-cholin causing an equal fall of pressure (fig. 8, p. 208 of preceding paper) and was frequently followed by a slow contraction. Histamin caused a pronounced diminution of the volume of the liver (fig. 20 of preceding paper, p. 224); it was not determined whether this was active or passive, due to the fall of blood pressure.

Mautner and Pick (65) state that there is in the liver of the dog and cat, either in the end capillaries of the portal or of the hepatic vein or in the area between these, a nervous mechanism which responds with a cramplike contraction to histamin; this, according to these authors, interferes with the return of the venous blood to the heart and leads to the fall of blood pressure. I found, however, that histamin causes a greater fall of pressure (cat) when injected into a saphena vein than when injected into a branch of the portal vein; also that large doses of sodium nitrite or acetyl-cholin injected into a branch of the portal vein were without effect upon the histamin fall of blood pressure.

Yohimbine caused a fall of blood pressure after an amount of atropine which had abolished a greater fall of pressure from acetyl-cholin; whether the depressor action was diminished by the atropine was not clear, for both the atropine and yohimbine caused a long continued lowered pressure and whereas yohimbine continued to cause the pressure to fall to the same level, or lower, both the absolute and percentile fall were less. In any case atropine has no action upon the yohimbine fall of pressure at all comparable to its action upon the effects of acetyl-cholin or pilocarpine. The latter was also true of *curare:* curare caused a fall of pressure after atropine but atropine diminished the fall somewhat. This antagonistic action of atropine towards curare had already been more carefully studied by Sollmann and Pilcher (66); they offer no explanation of the action.

Saline injection. Probably most experimenters are familiar with the fall of pressure which the intravenous injection of a small amount (1 or 2 cc.) of normal saline sometimes causes in certain animals but I have seen no discussion of it. I have observed this effect a number of times in cats but have made no systematic attempt to analyze it; the latter may prove difficult for, in my experience, the effect usually rapidly diminishes after a few injections and then can no longer be obtained. I have never observed this fall in the large number of experiments in which atropine had been injected; if it should be shown that atropine really does abolish the effect this would be of interest as suggesting that perhaps the same mechanism is involved as in the acetyl-cholin reaction. My own observations on this point are of practically no value for, with a single exception, the atropine was not injected until somewhat late in the experiment when the action, even if it had been present at all, would probably have spontaneously ceased. In the exceptional experiment to which reference was made the fall of pressure had been obtained ten times in succession in forty-five minutes and there was almost no indication that it was diminishing; about a minute after the last injection (1 cc. of a 0.9 per cent sodium chloride solution, which caused the pressure to fall from 107 to 80 mm.) 0.05 mgm. atropine sulphate was injected; the next injection of saline, made two and a half minutes later, caused only a very slight rise of pressure. A number of injections of saline were made during the next forty-five minutes but in no case was there a fall of pressure.

This saline fall of blood pressure was most frequently obtained in curarized cats or in cats anaesthetized with paraldehyde (1.7 cc. per K.); it occurred in animals in which the vagi, also in some in which the accelerators had been cut; it occurred not only with 0.9 per cent sodium chloride solutions but with Ringer's solutions of different formulas and with saline solutions freshly made with double glass distilled water and the purest (Kahlbaum) salts. It occurred in one animal in which a myocardiograph was attached to the right auricle; there was no weakening of the latter. The reaction seemed to be greater in animals especially susceptible to acetyl-cholin; and also in those in which the depressor action of epinephrin was pronounced. In one case the fall, which, as always, was of brief duration, was 51.1 per cent; usually it was between 20 and 35 per cent. Nearly all of the injections were made into a saphena vein; a few into a jugular. There was no change in the heart rate.

It is not improbable that this saline fall of pressure is analogous to

the flushings sometimes seen in sensitive individuals after intravenous injections of various kinds ("salvarsan," for example); possibly small doses of atropine would be found to have a prophylactic action.

Compounds related to cholin. The vasodilator action of cholin itself has been the subject of a number of investigations; among the most important of these were those of Mott and Halliburton (67), who found that the fall of pressure caused by cholin was replaced by a rise when atropine was injected, and of Müller (68) who made a further analysis of the action. The results of the studies with cholin have been somewhat contradictory (see (69), (70)) owing to the number of, often opposing, factors involved; no clear conception of the latter was possible until Dale's (1) work on the nicotine-like action of cholin on sympathetic ganglion cells but this does not clear up all points (e.g., the peripheral vasoconstriction after atropine found by Müller and evidently similar to that I have found with acetyl-cholin, and the apparent central action—sympathetic ganglion cell action?—described by Pilcher and Sollmann (71). As regards the vasodilator action of cholin: this has been less thoroughly investigated than that of acetyl-cholin but the action of the two seems to be qualitatively the same; as regards the quantitative results: Taveau and I in 1906 (72) found, in comparisons on the same animal, the acetyl compound to be about one hundred thousand times more active than cholin itself; in other experiments the difference has been even greater, much depending upon the anaesthesia and other factors.

I investigated, in collaboration with Taveau, seventy-nine compounds derived from cholin or analogous compounds (42), (72), (73); later, in collaboration with Menge (45), (74), (75), (76), I made a similar study of about twenty-five derivatives of the homocholins. Dale (1) has recently studied about a dozen other compounds related to this series. There is, therefore, a very large collection of data available for a consideration of the relation between the chemical composition and physiological action in this series.

While a detailed consideration of this subject belongs in a publication devoted to pharmacology and was discussed several years ago there are certain features which have a physiological interest. One of my general conclusions was that "the greatest effects upon the blood pressure are produced by those compounds which depart least from the choline type—that is, by those compounds containing the nucleus $HON\begin{matrix}(CH_3)_3 \\ CH_2CH_2O—\end{matrix}$; all changes in the group $(C_nH_{2n+1})_3$

or in the side chain (except the substitution of the terminal hydrogen atom) tended to diminish the effect upon the blood pressure but increased as a rule, the toxicity"—(there are exceptions to this as regards changes in the side chain); and further: "the greater the variation of the compounds from the cholin type, the less effect does atropine have in preventing a fall of blood pressure." These general principles were illustrated by the study of compounds containing ethyl, propyl, etc., groups in place of the methyl groups of cholin; in such compounds and their acetyl derivatives the acetyl-cholin type of action upon the blood pressure was absent or only slightly developed. An interesting illustration of the influence of methyl groups was seen in a comparison of the effects of the compounds $ClN\begin{matrix}(C_5H_{11})_3(iso)\\ CH_2CH_2OH\end{matrix}$ and $ClN\begin{matrix}(CH_3)_2\\ C_5H_{11}(iso)\\ CH_2CH_2OH\end{matrix}$; the former apparently had none of the blood pressure lowering property of acetyl-cholin whereas this was present, but to a less extent than with cholin itself, in the latter.

It is interesting to note in this connection that Launoy (77) found that compounds of the cholin type containing three methyl groups were far more active in stimulating pancreatic secretion than were analogous compounds containing ethyl, etc., groups.

I suggested that there may be a connection between the low toxicity of the cholin derivatives, as compared with the triethyl, etc., compounds, and their great physiological activity (when the H of the hydroxyl is substituted by appropriate groups, such as the acetyl group) and "the fact that cholin is a constituent of probably all plant and animal cells; these may have, to use the rather crude comparison of a mosaic (a figure of speech used by Ehrlich, one of the pioneer investigators in this field) places into which the compounds can fit and the cells themselves containing such groups are not injured by them as they would be by new and unusual groups;"[5] and further, "the rôle of this group is probably to carry the compounds to definite cell structures or, to use the comparison of Ehrlich, to make them fit in a certain mosaic; then the groups which have substituted the hydrogen atom enable the entire compound to exert a definite action (groups of the lower fatty series to depress the blood pressure, etc.)."

I also stated that "when this configuration is maintained it is pos-

[5] Joseph and Meltzer (78), found that the toxicity of magnesium, calcium, potassium, and sodium ions varies inversely to the proportion in which they occur in the serum of the animal.

sible to vary the intensity and character of action upon the circulation within extraordinarily wide limits by substituting groups in the place of the hydrogen atom" (of the hydroxyl). I illustrated this by substituting not only the acetyl but a large number of other acyl groups and found that the homologues of acetyl-cholin (propionyl-cholin, etc.) had the acetyl-cholin type of action upon the blood pressure, but that "as we pass up the series from the acetyl to the valeryl compounds we find a rapid diminution in the activity of these compounds in causing a fall of blood pressure" and an increase in their activity in other directions. The introduction of residues of the aromatic acids caused a still further departure from the acetyl-cholin type. Dale found that some of the simple esters and ethers of cholin had the acetyl-cholin type of action, but that none equalled this in intensity.

Another point of perhaps some physiological interest is the method employed to obtain a compound having the effect of acetyl-cholin upon the blood pressure but one with a more prolonged action and which would be sufficiently stable to be effective in small doses when given by mouth or injected subcutaneously. Taveau and I had noticed that acetyl-cholin is not very stable, undergoing hydrolysis somewhat readily, an observation since made by Ewins (79) and Fourneau and Page (80) and the physiological importance of which has been emphasized by Dale. It seemed possible that the compound might be rendered more stable by the substitution of certain groups in the side chain without destroying its physiological activity; it was these considerations which led Menge and me to take up the study of the homocholins with the result that Menge prepared the previously unknown α-methyl-cholin. The acetyl derivative of this compound has the physiological properties desired; it was so stable that a single subcutaneous injection of a few tenths of a milligram would keep the blood pressure uniformly lowered to one-half for hours. This low pressure could be almost instantly released, and to any desired extent, by the injection of atropine. This acetyl-α-methyl-cholin underwent hydrolysis less readily than did acetyl-cholin which probably accounts for its greater activity when injected subcutaneously or given per os. On the other hand certain other esters the chemical constitution of which led to the expectation that they would be very active were found not to be so, and Menge found these to be still more resistant to hydrolysis; thus the great activity of acetyl-cholin when injected intravenously seems to be connected with its instability. (Many interesting relations between the resistance of acetyl-cholin and some other esters and

ethers of cholin to the hydrolytic action of the blood, and, possibly, to the action of an esterase, and the action of these compounds upon various organs were discussed by Dale).

The introduction of the methyl group into acetyl-cholin caused a similar change in its action on the pupil: whereas acetyl-cholin applied to the cornea had no effect upon the pupil and even when injected intravenously caused but a brief constriction, acetyl-α-methyl-cholin applied to the cornea caused such a pronounced constriction as to suggest the possibility of its practical use as a miotic.

5. SUMMARY AND DISCUSSION

The above experiments, and those of the preceding paper, show that there is widely distributed in at least certain animals a vasodilator mechanism which has not hitherto been clearly recognized in physiology, pharmacology or pathology; the outstanding features of it are (1) its ability to respond, with great energy, to a limited group of compounds of the cholin type and to pilocarpine and perhaps a few others; (2) that this action is prevented by atropine; and (3) that it apparently is not connected with any of the known vasodilator nerves. It seems appropriate to speak of a "mechanism," for different blood vessels react unequally, i.e., something other than the contractile elements of muscular tissue is involved, and because the action is prevented by atropine; the latter reaction is usually interpreted as indicating the presence of a "nerve-ending" or of a "receptive substance" i.e., some kind of a "mechanism."

The compound to which this vasodilator mechanism responds most typically, or energetically, acetyl-cholin, has been taken as the typical parasympathetic nerve stimulant; Gaskell (4) speaks of the "acetyl-cholin group" of nerves (although, since most of these actions had been previously discovered in connection with muscarine the term "muscarine action" is more appropriate). As has been shown, especially by Dale, the action of this group "may be summarized, with but small qualification, as a reproduction of the effects of stimulating nerves belonging to the cranial and sacral divisions of the involuntary (autonomic) system." As pointed out by Dale, and investigated in more detail in my experiments, these compounds cause vasodilation not only in areas supplied by cranio-sacral vasodilators but in areas in which the existence of such nerves is anatomically not probable. An equally important qualification to the identification of the acetyl-

cholin vasodilator effect with that of parasympathetic nerve is the difference in the effects, in the two cases, of atropine: atropine readily abolishes the vasodilator action of acetyl-cholin in the regions of pelvic and chorda tympani nerve innervation whereas it has no effect upon the vasodilation caused by stimulating the former nerve and affects that of the latter only in very large doses. For the same reason it is impossible to identify other vasodilator actions of acetyl-cholin with that of the action of other parasympathetic or of any other vasodilator nerves: the effect of the drug is everywhere prevented by atropine, whereas that of nerve stimulation is nowhere so affected.

On the other hand the analogy between the action of acetyl-cholin and that of parasympathetic nerve stimulation upon so many organs is so striking that one is tempted to think of the vasodilator mechanism upon which acetyl-cholin acts as being constituted like the other mechanisms upon which it and the parasympathetic nerves act. It might be supposed that this mechanism has developed but has not become connected with nerves just as Dale and Laidlaw (81) suggested might be supposed to be the case with the uterus (which responds, when isolated, with contraction to pilocarpine and also, though feebly, to acetyl-cholin which actions are inhibited by atropine although there is no evidence that this organ receives a parasympathetic innervation). There may be no "need" for an extension of the parasympathetic nerves to the blood vessels just as there may be no "need" for their extension to the ventricle of the mammalian heart although this occurs in the frog. Especially interesting in this connection is the purely negative inotropic action upon the mammalian auricle which can be obtained with acetyl-cholin; the extent of the beat may be reduced almost to the vanishing point without there being any slowing. In the case of the auricle the substance upon which the drug acts is connected with nerves, whereas the similarly constituted substance in the blood vessels seems not to be connected with nerves. Possibly the substance in the blood vessels responds to the stimulus of some unknown hormone whereas that in the auricle is utilized for the more rapid adjustments under the nervous system. Of course the existence of similar mechanisms not intimately connected with nerves is not without analogy; one instance is the case of the uterus cited above, others are the intestines and urinary bladder: these are stimulated by pilocarpine and other "parasympathetic nerve stimulants" and the action of these is prevented by atropin whereas the effects of the vagus and pelvic nerves upon these organs, although the same as that of the

drugs, are not greatly modified by atropine. There are, however, very obvious differences between the two cases; the known vasodilator nerves can not for the most part be considered analogous to the vagus or pelvic nerves.

Of course it may be argued, as has been done in connection with the dilator action of pilocarpine, and of epinephrin on the salivary glands, that the vasodilator action of acetyl-cholin is due primarily to a stimulation of metabolism and that the dilatation is really due to certain products of metabolism. Such an argument may be applied with some plausibility to the dilator effect of acetyl-cholin upon the salivary glands which it also excites to increased activity. In fact a compound analogous to acetyl-cholin might be suggested as one of the "metabolites" found by stimulation of the chorda tympani to which the "vasodilator" action of this nerve has been attributed; if the cholin in the blood (50) which passes through the salivary glands, or possibly the minute amount which occurs in saliva, were present in the gland in the form of some of its esters or ethers these compounds might account for at least a part of the vasodilation following chorda tympani stimulation. But there is no warrant for suggesting that most of the vasodilator actions of acetyl-cholin are the result of increased metabolic activities.

Gaskell has suggested two "reasons" for the existence of vasodilator mechanisms: one to bring more blood to an active organ, another to regulate the blood pressure for the benefit of the heart. It has not been possible to show that the acetyl-cholin mechanism serves either of these functions; no evidence of any kind could be found that it is involved in the action of the depressor or of other nerves In fact, so far as has been determined, the mechanism seems as useless to the body as the host of receptors for various toxins, etc., postulated by Ehrlich; it may, however, be that it was evolved in connection with the metabolism of one of the constant constituents of the body, cholin.

The fact, however, that as yet no "function" can be ascribed to this vasodilator mechanism does not preclude the possibility that it may be utilized for therapeutic purposes on the one hand and, on the other, that it may be involved in pathological processes. It is generally believed that the body possesses, in the depressor nerve and its connections, a remarkable arrangement for the protection of the heart although it has never been satisfactorily shown how or when this may be called into activity; no suggestion has been made as to how any use of it for therapeutic purposes could be made. By the injection of

some of the cholin, and especially of the homocholin derivatives it is possible to control the blood pressure of an animal as effectively as by stimulation of the depressor nerve; possibly they may be found of therapeutic value not only in relation to general blood pressure but also in connection with local spasmodic contraction of blood vessels.

Whether this mechanism is involved in any pathological conditions is also unknown. It is conceivable that it is maintained in a condition of continued activity by products of metabolism and that its failure may be involved in some of the unexplained cases of hypertension. The fact that atropine, which prevents the action of cholin compounds upon the blood vessels does not ordinarily, unless perhaps, when the vagi are in a condition of great activity, cause a rise of blood pressure does not necessarily show that this mechanism is not in a condition of continued activity; atropine itself has a dilator action upon blood vessels (fig. 2, p. 242; also fig. 10, p. 212 of preceding paper) and moreover there are many compensating factors tending to maintain a normal blood pressure. On the other hand it may be that in some of the obscure pathological conditions in which atropine has been reported to be of value there is an abnormal activity of this mechanism.

CONCLUSIONS

The mechanism involved in the vasodilator action of acetyl-cholin and related bodies is different from that involved in the action of any of the nerves (posterior root, parasympathetic and sympathetic) to which vasodilator functions have been attributed. It is also different from that involved in the depressor action of epinephrin.

This mechanism, although capable of more energetic response than any hitherto described, is not involved in the action of the depressor or of other afferent nerves causing a fall of blood pressure.

The only substances found having the same type of vasodilator action as acetyl-cholin were a limited number of compounds derived from, or closely related to cholin and pilocarpine and colchicine.

Atropine and closely related substances were the only compounds found having a pronounced antagonistic action to the vasodilator action of acetyl-cholin. Pilocarpine diminished the action slightly. Physostigmine intensified all of the actions of acetyl-cholin.

BIBLIOGRAPHY

(1) Dale: Journ. Pharm. Exper. Therap., 1914, vi, 147.
(2) Barcroft: Respiratory function of the blood, 1914, 146.
(3) Bayliss: Principles of general physiology, 1915, 700.
(4) Gaskell: The involuntary nervous system, 1916.
(5) Langley and Anderson: Journ. Physiol., 1895, xix, 107.
(6) Piotrowski: Pflüger's Arch., 1894, lv, 240.
(7) Heidenhain: Pflüger's Arch., 1872, v, 309.
(8) Henderson and Loewi: Arch. Exper. Path. u. Pharm., 1905, liii, 62.
(9) Bayliss: Journ. Physiol., 1901, xxvi, 173; 1902, xxviii, 276. The older literature is given here.
(10) Ostroumoff: Pflüger's Arch., 1876, xii, 219.
(11) Pick: Arch. Exper. Path. u. Pharm., 1899, xlii, 399.
(12) Stevenson and Reid: Johns Hopkins Hosp. Bull., 1915, xxvi, 31.
(13) Carlson: This Journal, 1907, xix, 408.
(14) Dastre and Morat: C. R. Acad. d. Sci., 1880, xci, 393.
(15) Langley and Dickinson: Proc. Roy. Soc., 1890, xlvii, 379.
(16) Dale: Journ. Physiol., 1906, xxxiv, 163.
(17) Barger and Dale: Bio-Chem. Journ., 1907, ii, 240.
(18) Dale: Journ. Physiol., 1913, xlvi, 291.
(19) Sollmann and Brown: Journ. Amer. Med. Assoc., 1905, xlv, 229.
(20) Meltzer and Meltzer: This Journal, 1903, ix, 261.
(21) Pilcher and Sollmann: Journ. Pharm. Exper. Therap., 1915, vi, 339.
(22) Hartmann and Fraser: This Journal, 1917 xliv, 453.
(23) Cannon and Lyman: This Journal, 1913, xxxi, 376.
(24) Chiari and Fröhlich: Arch. Exper. Path. u. Pharm., 1911, lxiv, 214.
(25) Cushny: Journ. Physiol., 1908, xxxvii, 130.
(26) Bayliss: Journ. Physiol., 1893, xiv, 303; 1908, xxxvii, 264; Proc. Roy. Soc., London, 1908, 80 B, 339.
(27) Martin and Stiles: This Journal, 1914, xxxiv, 106.
(28) Tschirwinsky: Centralb. f. Physiol., 1895, ix, 777; 1898, x, 65.
(29) Howell, Budgett and Leonard: Journ. Physiol., 1894, xvi, 298.
(30) Hunt: Journ. Physiol., 1895, xviii, 393.
(31) Vincent and Cameron: Quart. Journ. Exper. Physiol., 1915, ix, 45.
(32) Martin and Lacy: This Journal, 1914, xxxiii, 212.
(33) Ranson and Billingsley: This Journal, 1916, xlii, 16.
(34) Gruber: This Journal, 1917, xlii, 214.
(35) Stiles and Martin: This Journal, 1915, xxxvii, 94.
(36) Martin and Stiles: This Journal, 1914, xxxiv, 106; ibid., 220.
(37) Martin and Mendenhall: This Journal, 1915, xxxviii, 98.
(38) Martin and Stiles: This Journal, 1916, xl, 194.
(39) Ranson and Von Hess: This Journal, 1915, xxxviii, 128.
(40) Langley: Journ. Physiol., 1912, xlv, 239.
(41) Ranson and Billingsley: This Journal, 1916, xl, 571.
(42) Hunt and Taveau: Hygienic Laboratory Bull. no. 73, U. S. Public Health Service, 1911.
(43) Trendelenburg: Arch. Exper. Path. u. Pharm., 1913, lxxiii, 118.
(44) Gaisböck: Arch. Exper. Path. u. Pharm., 1911, lxvi, 398.

(45) HUNT: Journ. Pharm. Exper. Therap., 1915, vi, 477.
(46) MCCARTNEY: Journ. Pharm. Exper. Therap., 1917, x, 83.
(47) RANSOM: Journ. Pharm. Exper. Therap., 1917, x, 169.
(48) HUNT: This Journal, 1902, vi, 19.
(49) LOEWI: Arch. Exper. Path. u. Pharm., 1912, lxx, 323.
(50) HUNT: Journ. Pharm. Exper. Therap., 1915, vii, 301.
(51) LEVY: This Journal, 1916, xli, 492 (earlier literature here).
(52) HUNT AND SEIDELL: Hygienic Laboratory Bull. no. 47, U. S. Public Health Service, 1908.
(53) MARINE AND ROGOFF: Journ. Pharm. Exper. Therap., 1916, ix, 1.
(54) KOBERT: Arch. Exper. Path. u. Pharm., 1886, xxii, 77.
(55) BRODIE AND DIXON: Journ. Physiol., 1904, xxx, 476.
(56) DIXON: Journ. Physiol., 1903, xxx, 97.
(57) BAEHR AND PICK: Arch. Exper. Path. u. Pharm., 1913, lxxiv, 65.
(58) BEREZIN: Pflüger's Arch., 1914, clviii, 219.
(59) DIXON AND HALLIBURTON: Quart. Journ. Exper. Physiol., 1910, iii, 315.
(60) FRÖHLICH AND PICK: Arch. Exper. Path. u. Pharm., 1913, lxxiv, 107.
(61) SOLLMANN: Manual of pharmacology, 1917, 297.
(62) DIXON AND MALDEN: Journ. Physiol., 1908, xxxvii, 50.
(63) DALE AND LAIDLAW: Journ. Physiol., 1911, xliii, 182.
(64) BARGER AND DALE: Journ. Physiol., 1911, xli, 499.
(65) MAUTNER AND PICK: Münch. med. Wochenschr., 1915, 1141.
(66) SOLLMANN AND PILCHER: This Journal, 1910, xxvi, 233.
(67) MOTT AND HALLIBURTON: Phil. Trans. Roy. Soc., London, 1899, 191 B, 211.
(68) MÜLLER: Pflüger's Arch., 1910, cxxxiv, 289.
(69) SAMELSON: Arch. Exper. Path. u. Pharm., 1911, lxvi, 347.
(70) HANDOVSKY AND PICK: Arch. Exper. Path. u. Pharm., 1912, lxxi, 89.
(71) PILCHER AND SOLLMANN: Journ. Pharm. Exper. Therap., 1915, vi, 381.
(72) HUNT AND TAVEAU: Brit. Med. Journ., 1906, ii, 1788.
(73) HUNT AND TAVEAU: Journ. Pharm. Exper. Therap., 1909, i, 303.
(74) MENGE: Journ. Biol. Chem., 1911, x, 399.
(75) MENGE: Journ. Biol. Chem., 1912, xiii, 97.
(76) MENGE: Hygienic Laboratory Bull. no. 96, U. S. Public Health Service, 1914.
(77) LAUNOY: Journ. d. Physiol. et. d. Path., 1913, xv, 312.
(78) JOSEPH AND MELTZER: Journ. Pharm. Exper. Therap., 1909, i, 1.
(79) EWINS: Biochem. Journ., 1914, viii, 44.
(80) FOURNEAU AND PAGE: Bull. Soc. Chim., 1914, xv, 544.
(81) DALE AND LAIDLAW: Journ., Physiol., 1912, xlv, 1.

THE INFLUENCE ON THE THYROID OF ANASTOMOSIS OF THE PHRENIC AND CERVICAL SYMPATHETIC NERVES

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Received for publication December 8, 1917

These experiments represent an attempt to repeat the observations of Cannon, Binger and Fitz (1) on the effects of fusion of the anterior root of the phrenic nerve with the cervical sympathetic upon the thyroid function in cats. They state that symptoms resembling those of Graves' disease in man developed. There was marked tachycardia, loose movements of the bowels and falling of the hair. The animals were unusually excitable. The basal metabolism was greatly increased. The pupil was larger on the operated side. In one of the animals exophthalmos and respiratory hippus developed on that side. In one animal in which the symptoms were well developed (2), removal of the thyroid gland on the operated side stopped the progress of the disease. Whereas other animals had died within three months of the first appearance of the symptoms, this cat lived for seven months after the operation, when it was purposely killed. The conclusion is drawn that the symptoms were due to increased thyroid secretion, owing to the bombardment of the gland by impulses discharged along the phrenic synchronously with the respiratory discharge.

Troell (3), working mainly with cats and dogs, stated that he did not find any change in the pupil corresponding to the respiration. But he mentions that in the animals in which he looked for this, the pupil was still contracted and the nictitating membrane still forward on the operated side, indicating that the innervation had not yet been restored at the time the animals were killed. Accordingly it could not be expected that evidence of rhythmical discharges along the phrenic should be obtained on these animals.

Quite recently Burget (4) has reëxamined the question, with a negative result. The animals (cats and rabbits) after recovery from the operation were apparently normal in every respect.

Our experiments were made on ten cats. Early in 1916, the central end of the anterior root of the phrenic was sutured by a single stitch end to end to the cephalic end of the cervical sympathetic. One of the animals died one week after the operation from pneumonia, another one month after the operation from the common epidemic disease characterized by "snuffles," cough, sneezing, poor appetite, progressive emaciation and falling hair, which was prevalent at the time among our stock cats. Two of the cats were killed eight months after the operation on account of the same disease. One was lost after five months. The remaining five cats were in excellent health at the time they were killed (eight, eight and one-half, nine, eleven and one-half and twenty-one and one-half months, respectively, after the operation). Before the animals were sacrificed, the condition of the nerves above and below the site of the anastomosis was carefully tested by electrical stimulation. In all the ten animals systematic and frequent observations were made from the time of operation. In the eight which were kept for several months, the pupil on the operated side had regained equality with its fellow and the nictitating membrane was retracted to the same extent. One of the protocols is reproduced as a sample in condensed form.

Condensed protocol, cat 5, adult, female

January 29, 1916. Phrenic-sympathetic anastomosis made on left side. Between January 30, 1916 and November 17, 1917, when the animal was sacrificed, observations were made frequently during the first year and at less frequent intervals during the rest of the period. Within the first two or three months the contracted pupil on the operated side gradually became more like the pupil on the right (unoperated) side, equality being finally established and maintained until the animal was killed. Reactions to light and accommodation were equal in both eyes. At no time was respiratory hippus observed. Both eyes responded simultaneously and to the same extent to such stimuli as psychical disturbances. No evidence of any abnormal condition was at any time present. The cat became somewhat of a laboratory pet, feeding well and maintaining an excellent nutritional state. On March 12, 1916, she gave birth to five normal kittens. They were reared in and remained a long time about the laboratory. Towards the end of June, 1916, the animal again became pregnant; normal parturition.

November 17, 1917. Pupils equal, react equally to light, no change with respiration. Changes in the size of the left pupil are always accompanied by similar changes in the right. Anesthetized with ether—the pupils remain equal; the left nictitating appears slightly more forward than the right. The pupils react equally to light, as before the anesthetic. No respiratory hippus. Dissected down to the site of the anastomosis; found neuroma uniting the anterior root of

the phrenic with the cephalic end of the cervical sympathetic. The identity of the nerves was verified at autopsy. The various nerves above and below the neuroma were now stimulated repeatedly with a weak interrupted current, which could be just distinctly felt by the tongue. In all, about twenty stimulations were made. Stimulation of the sympathetic cephalad to the neuroma gave marked dilatation of the pupil and retraction of the nictitating. The same result was obtained on stimulating the anterior phrenic root central to the neuroma. In the meantime the phrenic root was not cut. Stimulation of the sympathetic caudad to the neuroma gave no effect on the eye unless the electrodes were very near the neuroma, when a slight dilatation of the pupil, but no retraction of the nictitating, was elicited. Stimulation of the neuroma itself always gave good dilatation of the pupil and retraction of the nictitating. These results were verified several times. Then the anterior root of the phrenic was ligated as high up as possible and cut central to the ligature. It was now again stimulated with the same result as before, namely, good pupil and nictitating reactions. Stimulation of the sympathetic caudad to the neuroma and cephalad to it, respectively, and stimulation of the neuroma gave the same results as before. On ligation and section of the anterior phrenic root it was observed that the left pupil became smaller than the right and remained smaller for the rest of the experiment. The thyroids were removed for histological examination: they were approximately equal in size and color, and appeared normal. The adrenals were normal in appearance and size; the left weighed, 0.212 gram, the right, 0.219 gram. Sections from the two thyroids, hardened and stained in the same manner, showed an identical histological picture, that of normal thyroid.

RÉSUMÉ OF RESULTS

In none of the animals were any symptoms resembling those of Graves' disease observed. In several it was proved by electrical stimulation that functional union had occurred between the phrenic and the cells of the superior cervical ganglion innervating the iris and the nictitating membrane. It was shown in several of the cats that a tonic dilator effect must have been exerted through the phrenic on the pupil of the operated side. This follows from the fact that in animals in which it was demonstrated by electrical stimulation that the phrenic caused dilatation of the pupil, while the sympathetic below the neuroma did not, the pupils on the two sides were equal. In the cat which was allowed to live longest, it was proved directly by nerve section that the phrenic was exerting a tonic dilator effect, for when the anterior phrenic root central to the neuroma was divided the pupil on that side became smaller than the other and remained smaller. In several of the cats the effect of stimulation of sensory nerves (central end of cut sciatic) on the pupil was noted. Dilatation occurred on the operated side at the same time as on the normal side and in the same

degree. In none of the animals was respiratory hippus seen although carefully and repeatedly looked for. If the dilator innervation is due to impulses from the respiratory center, there does not seem to be any obvious reason why a rhythmical change synchronous with the respiration should not be present. We can only state that it was not seen in any of our cats. Exophthalmos did not develop in any of the animals. The thyroids were always approximately equal on the two sides and of the same color on gross examination. Histologically no difference was observed between the thyroid on the operated and that on the normal side. Variations in the weight of the adrenals were within the normal range.

BIBLIOGRAPHY

(1) Cannon, Binger and Fitz: This Journal, 1914, xxxvi, 363.
(2) Cannon and Fitz: This Journal, 1916, xl, 126.
(3) Troell: Arch. Int. Med., 1916, xvii, 382.
(4) Burget: This Journal, 1917, xliv, 492.

OBSERVATIONS ON THE POSTURAL ACTIVITY OF THE STOMACH

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Received for publication December 11, 1917

In a paper recently published Sherrington (1) summarizes our knowledge concerning tonus, both of smooth and of striated muscle. He points out that the definitions of tonus heretofore advanced are unsatisfactory inasmuch as some are ambiguous while others serve only to account for certain phases of muscular activity.

The muscle fiber, he believes, is not to be regarded as an elastic string for it has the property of exhibiting different lengths while exhibiting one and the same degree of tension.

> Unstriated muscle, like skeletal muscle, evidently functions for two main purposes which in some ways it is possible and desirable to consider apart. Of these one is the performance of movements which overcome resistance by the development of tension; the other is the adjustment of contractile length without necessarily any alteration of mechanical tension.

Visceral tonus, in this sense, connotes "postural activity."

The investigations reported in this paper were undertaken with the idea of studying, in more or less detail, the postural activity of the gastric musculature.

Mosso and Pellacain (2) were among the first to observe that the internal pressure of the bladder in man and in the dog might be practically zero when the viscus was empty and when it was full. On the other hand, a considerable degree of pressure might develop in the presence of only a small amount of fluid.

Somewhat similar observations were subsequently made on the stomach by Müller (3), Moritz (4), Kelling (5) and Sick and Tedesko (6). Müller found that in rabbits the intragastric pressure remained constant during the period in which the chyme passed through the pylorus into the small intestine. And in man Moritz noted no appreciable changes in pressure during the period of gastric digestion.

In the course of some investigations concerning the state of tension

of the abdominal, the gastric and the intestinal walls Kelling found that within certain limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus. The administration of morphine, chloral, chloroform or large quantities of ether, however, partially or completely inhibited the activity of the mechanism responsible for this compensation. Light narcotization with ether, on the other hand, did not appear to interfere with the reaction. Kelling concluded that the tonus of the stomach is an expression of the activity of the intrinsic nervous mechanism and that it is more or less independent of the central nervous system.

Sick and Tedesko observed that the gradual filling of a cat's stomach was not accompanied by any rise in internal pressure. The stomach, they thought, might be compared to the heart, the fundus resembling the auricles and the pyloric part the ventricles. The active relaxation of the musculature of the fundus which accompanies the entrance of food into the stomach Sick (7) regarded as the active diastole of the viscus.

While a number of investigators have thus noted the ability of the stomach to accommodate its capacity to a given load without exhibiting any appreciable rise in intragastric pressure, none of them appear to have regarded this form of receptive relaxation as an actual expression of reflex tonus, i.e., postural activity (Sherrington). The prevailing view seems to have been rather that this response on the part of the stomach is brought about by a lowering of the level of gastric tonus, meaning by tonus a state of tension in the gastric musculature. Sick, for example, adopting the hypothesis of v. Uexküll held that the distension of smooth muscle rests upon a compromise between the condition of excitation of the muscle and the load. A persisting condition of excitation (Erregungszustand) in the muscle v. Uexküll (8) termed tonus.

METHODS

Both rabbits and cats were used in the course of the experiments. In order to follow the changes in the postural configuration of the stomach the empty viscus was slowly filled with warm physiological saline solution. The fluctuations in the intragastric pressure were taken as an index of the adjustment of the smooth muscle to its new conditions.

When the observations were conducted on living animals a tube was introduced into the pyloric end of the stomach. This was connected

by means of a two-way stopcock with a chloroform manometer and a reservoir of salt solution. The latter was so arranged that it could readily be elevated or lowered to any desired level. After closing the oesophagus with a ligature the body of the animal was submerged in a bath of physiological saline solution the temperature of which was maintained at 38°C. by means of electric light bulbs. Interposed between the tube entering the stomach and the stopcock mentioned above was a glass coil which was also submerged in the bath. This served to warm the fluid entering the stomach.

In certain experiments the pylorus was ligated and the inlet tube made to enter the oesophagus. This reversal of the direction of the fluid entering the gastric cavity did not appear to affect, materially, the behavior of the stomach. Ultimately it was found that for the purposes of a comparative study (in the cat) additions of 5 cc. of fluid made every minute afforded the most satisfactory rate of increase of gastric contents. While a number of experiments were prolonged until the stomach ruptured, in order to determine the limits of muscular relaxation, in most instances the observations were discontinued some time previous to this.

Manometric readings were made with each addition of fluid. The values were then plotted. In the accompanying charts representative curves are presented which illustrate graphically the postural activity of the gastric wall under the conditions imposed by the experiments. Figure 1 is plotted on a different scale from that used in the remaining figures since in this experiment the additions of fluid were continued until the viscus ruptured.

The animals were all anaesthetized with ether. After being placed in the bath, they were kept in the primary stage throughout the periods of observation. Fairly deep anaesthesia, however, did not appear to interfere with the postural activity of the stomach.

When the vagi were used a cannula was introduced into the trachea. The nerves were then transected in the neck. The splanchnics were reached externally through a lumbar incision on either side.

Where observations were made on the excised stomach the viscus was connected with the manometer and reservoir, and then either immersed in the warm saline bath or placed in a moist chamber.

No attempt was made to ascertain the exact pressure in the normal starving stomach. Such determinations involve certain difficulties which it did not seem advisable to meet, since the objects of this study had nothing to do with the empty viscus.

During the preparation of the animal care was exercised to prevent the entrance of air into the stomach. A few centimeters of fluid were permitted to flow into the viscus. By adjusting the manometer the intragastric pressure was brought to zero. The experiment was then started.

The part played by the abdominal wall in these experiments is naturally not a negligible one. Even though the gastric musculature exhibits a compensatory relaxation with each addition of fluid the intragastric pressure must rise unless there is a compensatory increase in the capacity of the abdominal cavity. Such changes in the abdominal musculature have recently been demonstrated by Pike and Coombs (9). They believe that their experiments show that the regulation of the length of the abdominal muscles corresponding to the increase in volume of the contents of the abdominal cavity is a reflex process which falls into line with the other known instances of postural activity of muscle and nerve.

Care was exercised in the experiments conducted with the abdomen closed not to interfere with this reflex. In each instance, moreover, the experiment was repeated on another animal in which the abdominal wall had been widely opened in the saline bath.

RESULTS AND DISCUSSION

Normal postural activity. Figure 1 gives a graphic record of the postural adaptation of the stomach of the rabbit to slow increments in the volume of its contents. In the experiments represented by curves *A* and *B*, 2.5 cc. of saline solution were allowed to enter each stomach every two and one-half minutes until the viscus ruptured. Curve *A* shows a rather marked fluctuation in the degree of intragastric pressure up to near the conclusion of the experiment. This was probably due, in part at least, to peristaltic contractions—the animals had been starved for two days.

The striking feature of curve *A*, however, is the low average pressure which was maintained throughout the greater part of the experiment. The intragastric pressure only rose abruptly shortly before the organ ruptured. In contrast to this, curve *B* is shown. It represents the changes in pressure which occurred in an excised stomach.

The changes observed in the course of approximately similar experiments conducted on cats are pictured in curves *A* and *B*, figure 2 and curve *A*, figure 3.

In certain experiments fluid was withdrawn and re-introduced several times in succession. As compared with the level of intragastric pressure noted during the course of the primary filling, the levels during the second and third fillings were somewhat higher in some instances and a little lower in others. In general the same nice adjustment of the configuration of the viscus to its load was manifest.

Kelling (5) and Sick and Tedesko (6) found that within certain

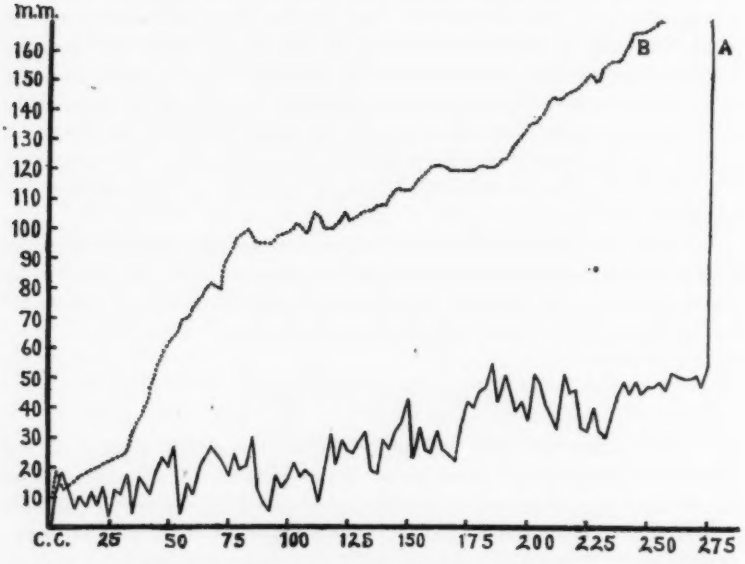

Fig. 1. *A*. Curve of intragastric pressure in a living rabbit; 2.5 cc. added every two and one-half minutes. Abdomen closed. *B*. Curve of intragastric pressure in an excised rabbit stomach immersed in physiological saline solution at 38°C., 2.5 cc. added every two and one-half minutes.

limits the intragastric pressure remained unaffected by the quantity of fluid within the viscus. By this it might be inferred that the relaxation of the walls becomes complete after each addition to the contents. It is probable, however, that in their work small increments of pressure were disregarded. In the fifty odd animals used throughout the course of this work no exception was found to this rule that any considerable increase in volume of contents was accompanied by a measurable rise in pressure. Often this was masked temporarily by the

effects of peristalsis; at other times a temporary drop took place, the exact explanation of which remained obscure though it suggested certain experiments of Rogers and Hardt (10) in which they observed that both in man and in the dog the fundic end of the stomach, during normal digestion, exhibited a slow tonus rhythm.

These results are in no way conflicting with our knowledge of the physiology of the gastric musculature. Cannon (11) has shown that "gastric peristalsis is augmented or weakened, is caused to appear or disappear as the tension on the contents is increased or decreased." And Alvarez (12) who places the seat of origin of the waves at a higher level in the stomach (cardia) believes that they may deepen into appreciable peristalsis at the place where the conditions defined by Cannon are right. It seems that a certain low level of intragastric pressure is essential to the motility of the stomach.

These conditions, then, serve to explain why the postural relaxation of the stomach seldom appears to be complete. A certain low degree of tension—dependent, in part at least, upon the volume of the contents—prevails so long as the viscus functions in the process of digestion.

Kelling (5) noted that time is an essential factor in what he termed the regulation of the intragastric tension. When the viscus was rapidly filled there was comparatively little compensatory relaxation of the walls. Curve *D*, figure 2, pictures the range of intragastric pressure observed subsequent to the sudden introduction of 150 cc. of saline solution into the empty stomach of a cat. During the course of the first five minutes there was a progressive rise of pressure up to 42 mm. of chloroform, a level which is almost half again as high as that observed after the gradual introduction (one-half hour) of the same quantity of fluid (curve *A*, fig. 2). Following this point the pressure very slowly sank to 28 mm.

Splanchnics and vagi. Carlson (13) has shown that section of the splanchnic nerves increases gastric tonus and augments hunger contractions. Section of both the splanchnics and the vagi, on the other hand, leads to a permanent hypotonus, except under conditions of prolonged starvation. Gastric hunger contractions, nevertheless, do persist after isolation of the stomach from the central nervous system.

In cats Cannon (11) found that cutting the vagi caused a temporary loss of tonus and an absence or marked weakness of peristalsis. He concludes that,

their function is solely to set the gastric muscles in a tonic state, to make them exert a tension, so that they are as if stretched by the contents, and the result of this condition is peristalsis.

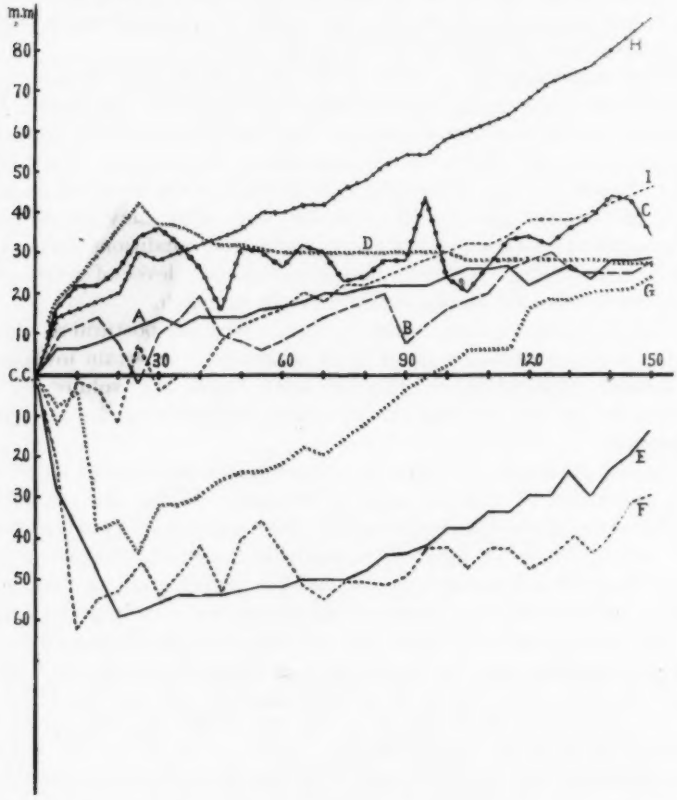

Fig. 2. *A*. Curve of intragastric pressure in a living cat; 5 cc. added every minute. Abdomen closed. *B*. Cat. Living. Abdomen open in physiological saline solution at 38°C., 5 cc. added every minute. *C*. Cat. Living. Abdomen closed. Splanchnic nerves divided; 5 cc. added every minute. *D*. Cat. Living. Abdomen open in salt solution; 150 cc. added at once. *E*. Cat. Living. Abdomen closed. Splanchnics and vagi divided; 5 cc. added every minute. *F*. Cat. Living. Abdomen open in salt solution. Splanchnics and vagi divided. *H*. Cat. Living. Abdomen closed. Splanchnics and vagi divided. 1 cc. 0.1 per cent solution of nicotine into jugular vein. *I*. Cat. Living. Abdomen open. Vagi divided.

Kelling (5) likewise concludes from his experiments that the vagi exert a very decided influence upon the tension of the gastric musculature. His results, however, were somewhat variable. He found that bilateral section might show no effect on the total tension of the viscus; again it might cause some decrease of tone, or it might raise it. Unilateral section (left) caused an increase of tonus which disappeared when the other vagus was cut. But these findings were not always the same.

Curve *C*, figure 2, affords a picture of the effects of bilateral section of the splanchnics on the postural activity of the stomach. The absence of the high tension which characterized the behavior of the excised stomach (curve *A*, fig. 3) pointed to the adaptation of the musculature to its burden. On comparing it with curve *A*, figure 2, it becomes evident that subsequent to the division of the nerves the pressure continued slightly more elevated throughout the entire period of observation. The fluctuations in pressure were due, in greater part at least, to exaggerated peristalsis.

The effects observed after section of one and of both vagi varied greatly from animal to animal. In the experiments represented by curves *I*, figure 2, and *F*, figure 4, the two vagi alone were divided; in those represented by curves *E*, *F* and *G*, figure 2, the splanchnics were also transected. From these curves it is apparent that the loss of the vagi results in no consistent change in the behavior of the musculature, so far as the postural activity of the organ is concerned. While the tension of the wall is observed to fall in certain instances (curves *E*, *F*, *G*, fig. 2), in others it rises to a level somewhat higher than that observed in the intact stomach (curve *I*, fig. 2). The fall in intragastric pressure, when it occurred, did not take place immediately subsequent to the section of the nerves. It appeared only as fluid was introduced into the stomach, during the process of accommodation in the musculature of the wall. The behavior of the wall here was very different from that of an inert, elastic body.

The development of a negative pressure in these experiments is explicable on mechanical grounds. In the course of the readjustment accompanying each increase in load, the absence of the pressor nerve fibers led to an exaggerated relaxation of the musculature, and this sufficed to lower the level of atmospheric pressure established at the outset of the observations. This of course does not imply that a negative pressure may develop in the stomach of an otherwise intact animal when the extrinsic nerves are sectioned, since normally there

exists a positive intragastric pressure. It simply illustrates how the degree of tension of the gastric wall, which characterizes the activity of the intrinsic nervous mechanism, may be influenced by means of its extrinsic nerve supply. Pressure changes of this character may be studied by means of a simple apparatus constructed from glassware and rubber dam. These changes in intragastric pressure, of course, are something distinct from the postural activity of the musculature.

The explanation of the differences in reaction among the animals is not clear. The findings, however, which are more or less consistent with those obtained by Kelling, serve to indicate that just as the essential characteristics of the hunger contractions of the empty stomach are determined by the local gastric motor mechanism rather than by the character of the central innervation (Carlson (13)), so the essential characteristics of postural activity are determined by the local neuro-muscular mechanism.

Cannon and Lieb (14) have shown that the inhibition which follows deglutition and leads to the receptive relaxation of the stomach is produced by way of the vagus nerves. From the considerations given above it is clear that a receptive relaxation may also take place in the absence of any extrinsic nerve supply.

Intrinsic nervous mechanism. In the course of their work on the law of the intestine Bayliss and Starling (15) found that both the local use of cocain and the intravenous administration of nicotine caused the true peristaltic movements to cease. The pendulum movements however persisted. This led them to believe that by the use of these drugs one might bring about a paralysis of the local nerve ganglia.

This conclusion has since been disputed, particularly by Magnus (16). The fact, however, as Gunn and Underhill (17) have pointed out, that the isolated intestine may continue to beat rhythmically in a solution of nicotine 1 to 1000 would suggest either that those movements are independent of nerve ganglia or that these ganglia are unusually resistant to nicotine.

Curve *C*, figure 3, furnishes a graphic record of the changes in intragastric pressure which were observed in an experiment in which the external surface of the stomach was painted with a 2.5 per cent solution of cocain. On comparing it with curve *A*, figure 2, (representing the reaction of a normal stomach) it became evident that the application of the drug led to a moderate increase in the tension of the gastric wall. This may have resulted from the local stimulating effects of the drug.

Curves *H*, figure 2, and *D*, figure 3, illustrate the changes in intragastric pressure observed following the intravenous injection of a large dose of nicotine (1 cc. of a 0.1 per cent solution into the jugular vein.) In one experiment, subsequent to the division of the vagi and splanchnics, the stomach was slowly filled with saline solution. Curve *E*, figure 2, furnishes a record of the changes in pressure noted during this period of the observations. The stomach was then emptied. After an interval of rest nicotine was injected intravenously and the

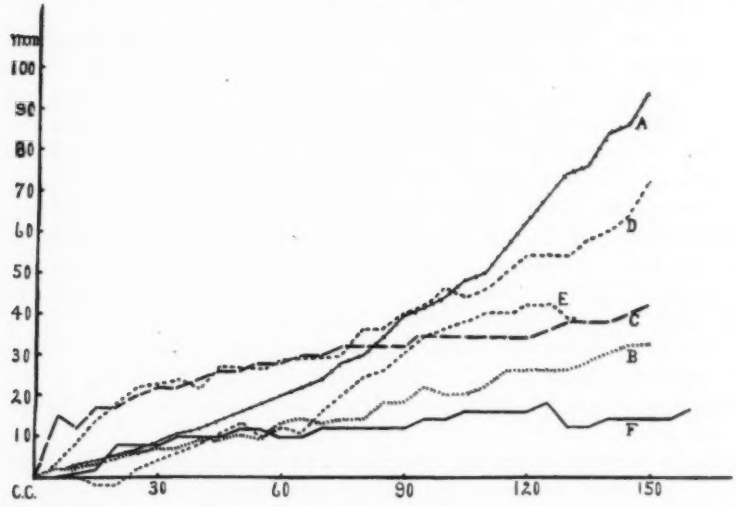

Fig. 3. *A*. Curve of intragastric pressure in an excised stomach (cat) in salt solution; 5 cc. added every minute. *B*. Cat. Living. Abdomen open. Pilocarpin $\frac{1}{120}$ grain. *C*. Cat. Living. Abdomen closed. Stomach rapidly painted with 2.5 per cent cocain solution. *D*. Cat. Living. Abdomen open. Splanchnics and vagi divided; 1 cc. 0.1 per cent solution of nicotine into jugular vein. *F*. Cat. Living. Abdomen open. Atropin $\frac{1}{60}$ grain in two doses.

filling process repeated. Curve *H*, figure 2, depicts the changes occurring in this half of the experiment.

The striking difference in the behavior of the muscle fibers in the two instances would appear to indicate that the drug had affected either the intrinsic nervous mechanism or the muscle cells directly. The fact, however, that the isolated intestine may continue to beat rhythmically in a solution of nicotine suggests that the latter possibility is the less likely.

The results from another experiment (not illustrated by a curve) also point toward the importance of Auerbach's plexus in the postural activity of the stomach. In this case the viscus was excised while the animal was in the primary stage of ether anaesthesia and placed immediately in oxygenated Locke's solution at 38°C. where it was kept throughout the period of filling. A curve plotted from the series of pressures observed corresponded closely to curve *A*, figure 3; that is, it showed an absence of the delicate adjustment of the musculature to its load which was noted in the normal stomach.

Gunn and Underhill (17) have demonstrated that strips of gastric musculature may continue to beat rhythmically for hours at a time under conditions of temperature and nutrition similar to these; and this occurs when the ganglia have been completely removed from the strips. The muscle cells of the gastric wall, in the presence of the necessary salts and oxygen, accordingly, may function independently of nervous influences. It seems fair to assume, then, that the imperfect relaxation of the walls in the experiment cited above was due, in part at least, to changes in the intrinsic nervous mechanism.

Curve *A*, figure 3, represents the effects on intragastric pressure of excising the stomach and keeping it in physiological saline solution at 38°C. throughout the period of the experiment. In curve *D*, figure 4, approximately the same changes are figured. The organ in the latter instance was kept in the ice chest for twenty-four hours before it was used.

Curve *B*, figure 4, on the other hand, shows a higher range of pressures, the result probably of a still greater interference with the postural activity of the muscle cells. The stomach, in this case, was kept for twenty-four hours at room temperature. This observation is in line with the experiences of Gunn and Underhill (17) who found that while the maximal time of survival of the intestine is somewhat over twenty-four hours when it is kept at 15°C., it is only about a third of that time when kept at 37°C. Alvarez (18) had much the same experience with specimens from the stomach.

A still poorer exhibition of postural adjustment in the freshly excised organ was observed when the stomach was kept in a moist chamber instead of in the usual saline bath (curve *C*, fig. 4.) This may have been due, in part, to vascular conditions since Hooker (19) has shown that the predominating effect of CO_2 upon the alimentary tract is to cause contraction of the muscle when in an arhythmic state. Curve *A*, figure 4, shows the effects observed from freely painting such a

viscus with cocain. In this concentration the drug probably acted as a general protoplasm poison.

Pilocarpin, atropin, adrenalin. Curve *B*, figure 3, pictures the results obtained with pilocarpin. The drug was injected immediately before the observations started in sufficient amounts to produce saliva-

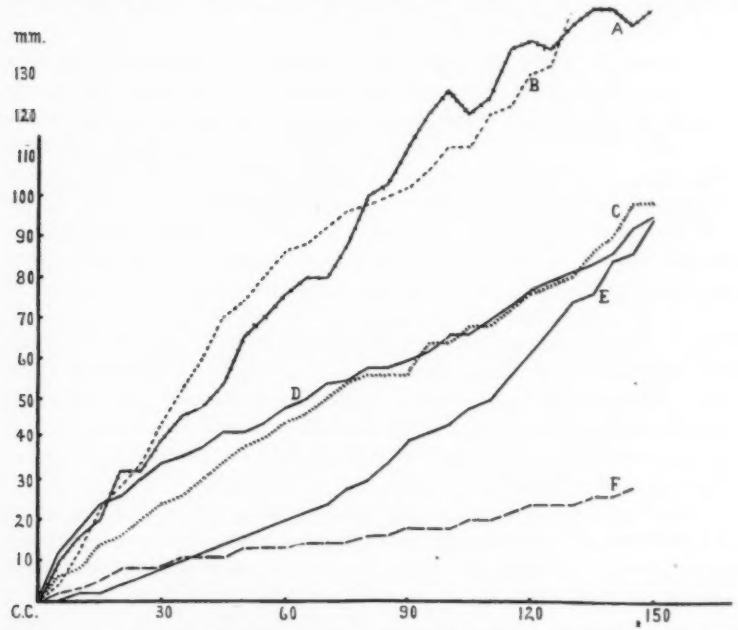

Fig. 4. *A*. Curve of intragastric pressure in an excised stomach (cat) kept in a moist chamber. Freely painted with 2.5 per cent solution of cocain. *B*. Excised stomach of cat in salt bath. Kept for twenty-four hours at room temperature. *C*. Excised stomach of a cat (fresh) in moist chamber. *D*. Excised stomach of a cat in salt bath. Kept for twenty-four hours in ice box. *E*. Excised stomach of a cat (fresh) in salt bath. *F*. Cat. Living. Abdomen open. Vagi divided.

tion. In general the reaction of the stomach was very similar to that noted when no drug was used (curve *B*, fig. 2). Larger doses, of course, in bringing about exaggerated peristalsis raise the intragastric tension considerably. Pilocarpin, it would appear then, exercises no direct influence on the mechanism responsible for postural activity; and affects it indirectly only when it is used in large amounts.

To study the effects of atropin the drug was injected both at the outset of the observations and also after 75 cc. of fluid had been run into the stomach (curve *F*, fig. 3). On the whole the musculature adapted itself to the increments of load with very slight changes in tension. The pressure at the conclusion of the work was approximately one-half that noted at a similar point in the experiment with a normal stomach (curve *B*, fig. 2).

Observations similar to this have led to the statement that atropin in proper dosage decreases gastric tonus. Atropin does lessen the tension of the gastric musculature through its action on the vagus terminals. Tonus or postural activity however, is something different from this. In eliminating certain extrinsic influences the drug nevertheless acts in a way which favors postural changes in the wall.

In the experiments with adrenalin 1 cc. of a 1: 10,000 solution was injected intravenously immediately before the first 5 cc. of saline were run into the stomach. Subsequent to the introduction of 50 cc. of fluid, 2 cc. additional of the drug were administered. Curve *E*, figure 3, illustrates such an experiment. For a few minutes following each injection of the drug there was a slight fall in intragastric pressure. This is suggestive of the experience of Cannon (11), namely, that the administration of a small dose of adrenalin will abolish intragastric pressure. Subsequent to each of these temporary depressions, however, the intragastric pressure rose rather rapidly.

The fall after each injection may be accounted for through the action (stimulation) of the drug on the depressor fibers of the sympathetic system in the gastric wall. The rises in pressure are less readily explained. There are several possibilities, however. The drug might lead eventually to a depression of the depressor fibers, or a heightened vagus action might follow after the drug had ceased to act.

CONCLUSIONS

The experiments outlined above were carried out in order to study, in some detail, the postural activity (Sherrington) of the gastric musculature. From the results of the work the following points may be emphasized.

The normal stomach possesses a striking capacity for adapting its size to the volume of its contents with only minimal changes in intragastric pressure. This capacity disappears only shortly before the viscus ruptures.

Time is an essential factor in the expression of this form of muscular activity—as the rate of change in volume of contents increases, the extent of the postural activity decreases.

The extrinsic nerves have nothing directly to do with the postural configuration of the viscus. The mechanism responsible for these changes concerns solely the musculature itself together with the intrinsic nervous mechanism.

Pilocarpin, atropin and adrenalin likewise have only an indirect influence upon postural activity. They neither increase nor decrease gastric tonus, but, like the extrinsic nerves, serve to regulate the tension of the stomach walls.

Excision of the stomach leads to a marked decrease in its postural activity, due in greater part probably to changes in the intrinsic nervous mechanism.

BIBLIOGRAPHY

(1) Sherrington: Brain, 1915, xxxviii, 191.
(2) Mosso and Pellacain: Quoted by Grutzner, Ergebn. d. Physiol., 1904, iii, 12.
(3) Müller: Quoted by Kelling, Zeitschr. f. Biol., 1903, xliv, 161.
(4) Moritz: Zeitschr. f. Biol., 1895, xxxii, 313.
(5) Kelling: Zeitschr. f. Biol., 1903, xliv, 161.
(6) Sick and Tedesko: Deutsch. Arch. f. klin. Med., 1908, xcii, 416.
(7) Sick: Deutsch. Arch. f. klin. Med., 1907, lxxxviii, 169.
(8) v. Uexküll: Ergebn. d. Physiol., 1904, iii, 1.
(9) Pike and Coombs: This Journal, 1917, xlii, 395.
(10) Rogers and Hardt: This Journal, 1915, xxxviii, 274.
(11) Cannon: This Journal, 1911, xxix, 250.
(12) Alvarez: This Journal, 1916, xl, 585.
(13) Carlson: This Journal, 1913, xxxii, 369.
(14) Cannon and Lieb: This Journal, 1912, xxix, 267.
(15) Bayliss and Starling: Journ. Physiol., 1899, xxiv, 99.
(16) Magnus: Arch. f. d. gesammt. Physiol., 1905, cviii, 1.
(17) Gunn and Underhill: Quart. Journ. Exper. Physiol., 1915, viii, 275.
(18) Alvarez: This Journal, 1917, xlii, 422.
(19) Hooker: This Journal, 1912, xxxi, 47.

THE RÔLE OF CATALASE IN "SHOCK"

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Received for publication December 12, 1917

Henderson (1) observed that the oxygen intake in "surgical shock" is decreased by about 45 per cent and that it is accompanied by a condition of acidosis. Presumably, therefore, oxidation must be decreased in the condition of "shock." We have shown that when oxidation is increased as for example in the excitement stage of anaesthesia, or in combat or by increasing the amount of work, there is a corresponding increase in catalase, an enzyme in the tissues which can liberate oxygen from hydrogen peroxide, and that when oxidation is decreased as for example by decreasing the amount of work, or in deep narcosis, or rendered defective as in pancreatic diabetes, there is a corresponding decrease in catalase. The object of the present investigation was to determine whether or not there is a decrease in the catalase of the tissues in "shock" corresponding with the decrease in oxidation. In view of the fact that catalase is decreased by the administration of anaesthetics and that the decrease is proportional to the depth of anaesthesia, precautions were taken in all the experiments reported in this paper to see that just sufficient ether was administered to keep the animal quiet and unconscious and that this amount was kept as uniform as possible. Determinations of the catalase of the blood were made in all the experiments during the administration of ether and previous to the production of "shock" to see that the catalase of the blood had become practically constant. When this was found to be the case "shock" was produced in the ways described and the catalase determined. Dogs and cats were used in the experiments. "Shock" was produced in the cats by exposure and manipulation of the intestines, and in the dogs by bleeding.

The cats were etherized and determinations of the catalase of the blood made and when these determinations were found to be constant, they were taken for comparison as the normal catalase content of the blood of the animal. The abdominal wall of the cat was then opened

by making a mid-ventral incision and the intestines were drawn out and handled. The catalase of the blood was determined at intervals of twenty minutes after the opening of the abdomen, as had been done previous to the opening of the abdomen. The blood pressure was taken in the carotid artery by means of a mercury manometer at the time of each determination of catalase. The catalase was determined by adding 0.5 cc. of blood to 250 cc. of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted graduated cylinder previously filled with water. After the volume of gas thus collected in ten minutes had been reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The bottles were shaken in a shaking machine during the determinations at a fixed rate of about one hundred and eighty double shakes per minute.

The curve in figure 1 was constructed from data obtained from a cat previous to the production and during the development of "shock." The lower figures (0 to 360) along the abscissa indicate time in minutes while the upper figures indicate blood pressure in millimeters of mercury. The figures along the ordinate (0 to 540) indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood taken from the external jugular vein. It may be seen that during the two 20-minute periods or 40 minutes, during the administration of ether but previous to the opening of the abdomen, 0.5 cc. of blood liberated 450 and 450 cc. of oxygen in ten minutes from hydrogen peroxide, and that the blood pressure was 105 and 103 mm. of mercury respectively; that during the succeeding five periods of 20 minutes each after the abdominal wall was opened and the intestines exposed, the blood liberated 440, 420, 420, 420 and 425 cc. of oxygen respectively, and that the blood pressure had fallen from 103 to 60 mm. of mercury. Hence during the five periods of 20 minutes each, or 100 minutes of exposure and handling of the intestines and administration of ether, the blood pressure had not fallen to a very low level, and there was a very small decrease in the catalase of the blood as is indicated by the small decrease in the amount of oxygen liberated from the hydrogen peroxide. During the succeeding ten periods of 20 minutes each, however, it will be noted that the blood pressure fell from 60 to 26 mm. of mercury and that during this time the catalase of the blood decreased by about 37 per cent as is indicated by a decrease in the amount of oxygen

liberated from 425 to 270 cc. It should be mentioned in this connection that the condition of the animal during the last two hours of the experiment was such that it was unnecessary to administer any ether,

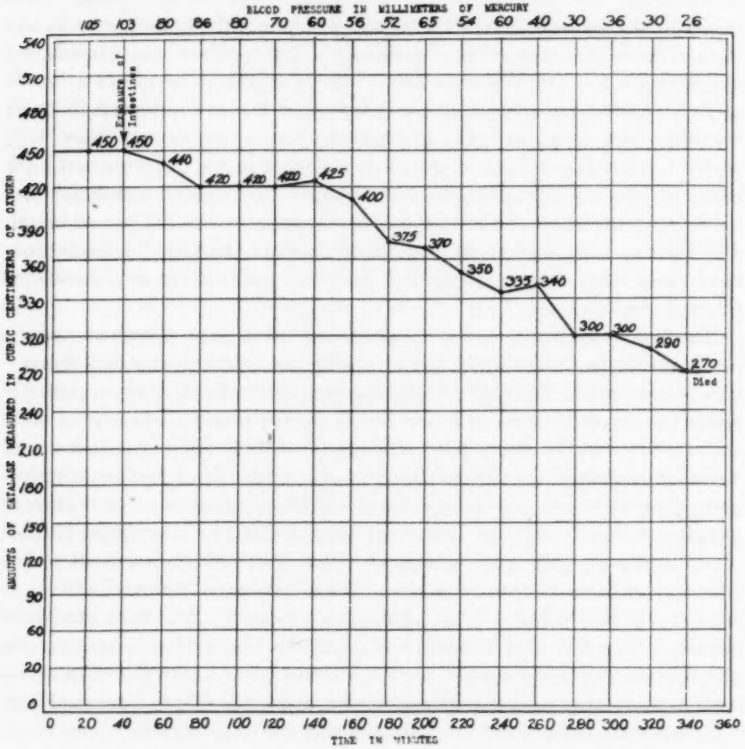

Fig. 1. Curve showing the effect of "shock" on the catalase content of the blood. The lower figures (0 to 360) along the abscissa indicate time in minutes, the upper figures along the abscissa indicate blood pressure in millimeters of mercury. The figures (0 to 540) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

hence the ether could not have played any part in this decrease in catalase. A discussion of the cause of the decrease in the catalase of the blood of the cats in the condition of "shock" will be given in the discussion of the results from dogs in this same condition.

Several dogs, weighing about 10 kgm. each, were bled approximately 5 per cent of the body weight. The blood was taken from the external jugular by means of a hypodermic needle attached to a flask in which a partial vacuum was created. Previous to being bled the dogs were slightly etherized, and during this period of slight anaesthesia determinations of the catalase of the blood were made. When these determinations were found to be constant, they were taken for comparison as the normal catalase content of the blood of the animal. The blood was then drawn from the external jugular at the rate of approximately 15 cc. per minute until 500 cc. of blood had been taken. After losing this amount of blood, the dogs were too weak to walk and would lie more or less quietly in apparently a semi-conscious condition. It was assumed that these animals were in the condition known as "shock." At the intervals given along the abscissa in chart 2, samples of blood were taken from the external jugular and the catalase content determined. The determinations were made in the same manner as those with the cat's blood except that 50 cc. of hydrogen peroxide were used instead of 250 cc. because of the low catalase content of the dog's blood.

Curves *a*, *b* and *c* in figure 2 were constructed from data obtained from dogs in the condition of "shock" produced as described. It will be seen that 0.5 cc. of the samples of blood taken from the different dogs during the administration of ether, but previous to the production of "shock," liberated 52, 48 and 42 cc. of oxygen respectively, and that two hours after the hemorrhage, when the animals were in the condition of "shock," the catalase had decreased by approximately 40 per cent as is indicated by the decrease in the amount of oxygen liberated to 32, 28, and 20 cc. respectively.

The explanation that suggests itself for the decrease in the catalase of the blood of the dogs and of the cats in the condition of "shock" is the decreased output of catalase from the liver and possibly the dilution of the blood by the diffusion of liquid, poor in catalase, from the tissues into the blood stream. The following observations are offered in support of the part played by the liver in this respect. The portal vein and the hepatic artery of a dog were tied off at 12.45 a.m. At 1.45 a.m. the blood pressure had fallen from 140 mm. of mercury to 22 mm. and the catalase of the blood had decreased by 50 per cent. Only the portal vein of another dog was tied off at 2.20 a.m. At 3.20 a.m. the same great fall in blood pressure was observed but the decrease in the catalase of the blood was not so extensive being only 30

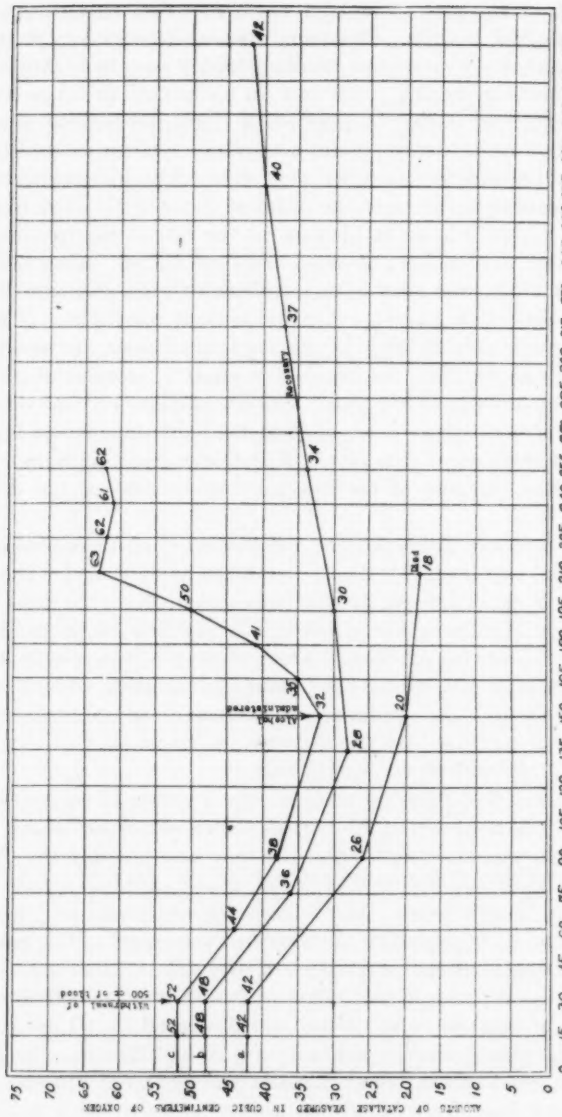

Fig. 2. Curves showing the effect of "shock" on the catalase content of the blood and the effect of the administration of alcohol. The figures (0 to 450) along the abscissa indicate time in minutes. The figures (0 to 75) along the ordinate indicate amount of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

per cent as opposed to the 50 per cent decrease when the entire blood supply was cut off from the liver. In the first case all the blood supplied to the liver being cut off, the output of the catalase from the liver was reduced to zero with the corresponding great decrease in the catalase of the blood. In the second case the blood supply being partially cut off, the liver continued to pour catalase into the blood with the resulting smaller decrease in catalase. We had found that when the liver was cut out of the circulation by an Eck fistula and by tying off the hepatic artery, the decrease in the catalase of the blood was not so great as when the portal vein as well as the hepatic artery was tied off with resulting decrease in general arterial pressure. This led us to the adoption of the second part of the explanation for the decrease in the catalase of the blood in the condition of "shock" namely, the dilution of the blood by the diffusion of liquid from the tissues. I know there is evidence that in "shock" there may be a diffusion of liquid from the blood into the tissues instead of from the tissues into the blood stream; however, as I have seen the evidence, the question seems to be an open one still. If there is a diffusion of liquid and the contained catalase from the tissues into the blood in "shock" this should produce a decrease in the catalase of the tissues. The catalase content of the different tissues of all the animals used in this investigation was determined and the results were found to be very irregular, although on the whole there was probably a decrease.

The dog from which data for curve *a* in figure 2 were obtained died about three hours after the production of "shock," when the catalase of the blood had been decreased by approximately 60 per cent as is indicated by a decrease in the amount of oxygen liberated from 42 to 18 cc. The condition of the dog from which data for curve *b* were obtained, began to improve about four hours after the production of "shock," and along with this improved condition, as may be seen in the chart, there was a gradual increase in the catalase content of the blood. Reasons will be given shortly for believing that the increase in catalase during the period of recovery from "shock," was due to the increased output of catalase from the liver. One hundred cubic centimeters of 40 per cent ethyl alcohol were introduced into the stomach of the dog from which data for curve *c* were obtained about three hours after the production of "shock." At the time of the introduction of alcohol it will be seen that the catalase had decreased by approximately 40 per cent from the normal as is indicated by a decrease in the amount of oxygen liberated from 52 to 32 cc. It may be seen

that the catalase of the blood increased very rapidly after the introduction of the alcohol, and that after one hour it had increased more than 90 per cent, as is indicated by the increase in the amount of oxygen liberated from 32 to 63 cc. In some unpublished results, we found that the introduction of alcohol into the stomach of an animal produced a very rapid increase in the catalase of the blood provided the liver was not excluded from the circulation, but that when this organ was excluded by an Eck fistula and by ligating the hepatic artery, alcohol produced very little or no increase. This observation was interpreted to mean that the great increase produced in the catalase of the blood by alcohol was due to the stimulation of the liver to an increased output of the enzyme.

In some unpublished results we found that the catalase of the liver was decreased in pancreatic diabetes by 72 per cent, and that of the heart by 48 per cent. In view of the fact that catalase content is so inseparably connected with oxidation in the body, the assumption was made that the defective oxidation in pancreatic diabetes was due to the decreased catalase in the tissues, this being brought about by the decreased output from the liver. Benedict and Török (2) found that the use of alcohol as a food in diabetes reduced the output of acetone, nitrogen and glucose. Neubauer (3) showed that red wine reduced the sugar output and the acidosis in diabetes. Allen and Dubois found that the administration of whiskey increased the oxidation of glucose in diabetes. From the results of these observers, it would appear that alcohol aids oxidation in diabetes. It may be that the helpful effect of moderate amounts of alcohol in diabetes is due to the stimulating effect of alcohol on the liver which causes an increased output of catalase from this organ and hence an increase of catalase in the blood and tissues thus facilitating the oxidative processes. If alcohol is helpful in diabetes, a disease in which there is a decrease in the catalase content of the tissues with resulting defective oxidation and acidosis, it would seem that it should be helpful in "shock" where these same conditions prevail.

SUMMARY

In the condition of "shock" the catalase of the blood and probably of the tissues is decreased. The decrease in the catalase of the blood is brought about by a diminished output of catalase from the liver owing to the lowered blood pressure and probably by a dilution of the blood due to a diffusion of liquid, poor in catalase, from the tissues.

The administration of alcohol in the condition of "shock" greatly increases the catalase of the blood and hence of the tissues by stimulating the liver to an increased output of this enzyme.

Since catalase content is so inseparably connected with oxidation in the body, the assumption is made that the decrease in catalase is principally responsible for the decreased oxidation with rsesulting acidosis in the condition of "shock," and that the beneficial effect of alcohol as a stimulant in "shock" and in conditions of general depression is due to the increase it causes in the catalase of the blood and tissues with resulting increase in oxidation and decrease in acidosis.

BIBLIOGRAPHY

(1) Henderson: Journ. Amer. Med. Assoc., 1917, lxix, 965.
(2) Benedict and Török: Zeitschr. f. klin. Med., 1906, lx, 329.
(3) Neubauer: Münch. med. Wochenschr., 1906, liii, 791.

SECRETIN

II. Its Influence on the Number of White Corpuscles in the Circulating Blood

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Received for publication December 18, 1917

In a previous report (1) we have shown that the subcutaneous injection of even a small dose of secretin is able to produce a marked increase in the number of erythrocytes in the circulating blood. In the present report we wish to show that such injections are likewise capable of increasing the number of white corpuscles in the blood stream.

The secretin which we used was in all cases prepared from the intestine of the dog. The mucous membrane was scraped off with a dull knife from the upper half of the small intestine, triturated with 50 cc. of 0.4 per cent hydrochloric acid and after standing for two hours was boiled actively. The preparation was neutralized while boiling and filtered. To it was then added sufficient glacial acetic acid to make 2 per cent by volume and the acid extract evaporated to dryness. We have found that such a preparation retains its activity for at least six months. We obtained about 10 mgm. of this dried acid extract per cubic centimeter of original solution. In all of the present series of experiments such an acid extract was used, a sufficient quantity of the dried preparation being dissolved in normal saline solution as needed to make a solution of the same strength as the original filtrate.

As in our previous experiments rabbits were used exclusively because it has been shown by Lamson (2) that they do not respond to fright, pain, etc., by an increase in the number of erythrocytes in the circulating blood, as do the cat and dog, and we wished to avoid the use of an anaesthetic. Also to exclude the factor of digestion leucocytosis food and water were withheld from the animals during the experiments.

The blood was obtained from the ear of the rabbit with as little manipulation as possible. Specimens were taken simultaneously for counting both white and red corpuscles, 0.5 per cent acetic acid being

used as the diluting fluid for the former and normal saline solution for the latter. The counts were made in the usual manner with the Thoma-Zeiss apparatus.

TABLE 1

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
1	W. B. C.	4,800	7,800	3,000	62.5	30	65
	R. B. C.	(*)					
2	W. B. C.	10,400	13,600	3,200	30.76	30	60
	R. B. C.	4,960,000	5,608,000	648,000	13.06	30	30
3	W. B. C.	9,600	11,250	1,650	17.18	90	90
	R. B. C.	5,331,000	6,240,000	909,000	17.05	90	90
4	W. B. C.	6,200	14,200	8,000	129.03	30	90
	R. B. C.	7,349,000	7,840,000	491,000	6.68	30	30
5	W. B. C.	11,600	16,600	5,000	43.10	60	90
	R. B. C.	6,054,000	7,184,000	1,130,000	18.66	60	60
6	W. B. C.	20,000	34,800	14,800	74.00	60	90
	R. B. C.	5,234,000	7,200,000	1,966,000	37.56	60	90
7	W. B. C.	15,600	11,786†	3,814	24.44	30	60
	R. B. C.	6,427,000	6,631,000	204,000	3.17	90	90
8	W. B. C.	12,654	15,800	3,146	24.86	60	90
	R. B. C.	6,615,000	7,760,000	1,145,000	17.30	60	60
9	W. B. C.	10,900	13,100	2,200	20.18	60	60
	R. B. C.	5,605,000	6,912,000	1,307,000	23.31	60	60
10	W. B. C.	7,400	12,200	4,800	64.86	60	90
	R. B. C.	5,343,000	6,246,000	903,000	16.90	60	60
Averages	W. B. C.	10,915	15,113	4,198	44.2	51	78.5
	R. B. C.	5,879,777	6,846,777	967,000	17.07	60	63.33

* Red corpuscle counts were not made in experiment 1.

† Experiment 7 shows a decrease in the white corpuscle count

Experiment 5, November 9, 1917

9.50 a.m. White blood corpuscles 11,600 per cubic millimeter. Red blood corpuscles 6,054,000 per cubic millimeter.

9.55 a.m. 1 cc. secretin solution (representing 10 mgm. of dried extract) per kilogram of body weight given hypodermatically.

10.25 a.m. White blood corpuscles 14,400 per cubic millimeter. Red blood corpuscles 6,496,000 per cubic millimeter.

10.55 a.m. White blood corpuscles 16,600 per cubic millimeter. Red blood corpuscles 7,184,000 per cubic millimeter.

11.25 a.m. White blood corpuscles 14,400 per cubic millimeter. Red blood corpuscles 5,984,000 per cubic millimeter.

11.55 a.m. White blood corpuscles 12,400 per cubic millimeter. Red blood corpuscles 5,216,000 per cubic millimeter.

12.25 p.m. White blood corpuscles 8,800 per cubic millimeter. Red blood corpuscles 5,574,000 per cubic millimeter.

We had determined in our previous experiments that 1 cc. of the secretin solution, equivalent to approximately 10 mgm. of the dried extract, per kilogram of body weight was the most efficient dose to produce an increase in the number of erythrocytes per unit volume of blood and that the preparation was effective when injected subcutaneously. Therefore we selected this dose as our starting point and administered it subcutaneously in all cases. Table 1 summarizes the results of ten such experiments, the effect upon both red and white corpuscles being recorded. Following the table is the protocol of a typical experiment of this group.

These experiments show conclusively that not only is secretin solution, when injected subcutaneously, able to produce an increase in the number of erythrocytes in the circulating blood but that it is capable of producing an even greater effect on the number of white blood corpuscles. In addition, however, it shows that the duration of the effect on the number of the corpuscles and the time of appearance of the maximum count are very nearly the same in the two cases—duration of effect on the red blood corpuscles 63.33 minutes, on the white corpuscles 78.5 minutes; maximum count of red blood corpuscles per cubic millimeter in 60 minutes, of white blood corpuscles per cubic millimeter in 51 minutes—the effect being produced quicker in the case of the white corpuscles and persisting longer.

We next sought to determine if the dose of 1 cc. of secretin solution per kilogram of body weight was the most efficient dose in the case of the white blood corpuscles as it had been shown to be with regard

to the erythrocytes. To do this we performed four experiments using in each a dose of ½ cc. of secretin solution per kilogram of body weight and four experiments using in each 2 cc. of secretion solution per kilogram of body weight. The results of these experiments are shown in tables 2 and 3 respectively. An experiment typical of each group is also given in detail.

TABLE 2

Dose: 0.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
11	W. B. C.	16,800	20,600	3,800	22.61	30	60
	R. B. C.	6,961,000	7,671,000	710,000	10.19	30	30
12	W. B. C.	9,200	10,600	1,400	15.21	60	90
	R. B. C.	5,440,000	6,560,000	1,120,000	20.58	60	90
13	W. B. C.	9,200	14,200	5,000	54.34	90	60
	R. B. C.	4,290,000	4,650,000	360,000	8.39	90	60
14	W. B. C.	16,800	18,400	1,600	9.54	60	60
	R. B. C.	6,640,000	6,928,000	288,000	4.33	60	60
Averages	W. B. C.	13,000	15,950	2,950	25.42	60	67.5
	R. B. C.	5,832,750	6,452,250	619,500	10.87	60	60.0

Experiment 11, November 20, 1917

11.05 a.m. White blood corpuscles 16,800 per cubic millimeter. Red blood corpuscles 6,961,000 per cubic millimeter.

11.15 a.m. 0.5 cc. secretin solution (representing 5 mgm. of dried extract) per kilogram of body weight given hypodermatically.

11.45 a.m. White blood corpuscles 20,600 per cubic millimeter. Red blood corpuscles 7,671,000 per cubic millimeter.

12.15 p.m. White blood corpuscles 18,400 per cubic millimeter. Red blood corpuscles 6,624,000 per cubic millimeter.

12.45 p.m. White blood corpuscles 16,000 per cubic millimeter. Red blood corpuscles 6,168,000 per cubic millimeter.

1.15 p.m. White blood corpuscles 14,500 per cubic millimeter. Red blood corpuscles 5,860,000 per cubic millimeter.

TABLE 3

Dose: 2 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
						minutes	*minutes*
17	W. B. C.	11,600	13,000	1,400	12.06	30	90
	R. B. C.	6,576,000	7,263,000	687,000	10.44	30	60
18	W. B. C.	11,700	19,400	7,700	65.81	60	90
	R. B. C.	4,960,000	5,900,000	940,000	18.95	30	90
21	W. B. C.	7,600	7,300*	300	3.94	40	80
	R. B. C.	6,001,000	7,313,000	1,312,000	21.86	30	30
22	W. B. C.	5,400	10,200	4,800	88.88	30	60
	R. B. C.	6,880,000	7,376,000	496,000	7.20	30	60
Averages	W. B. C.	9,075	12,475	3,400	38.21	40	80
	R. B. C.	6,104,250	6,963,000	858,750	14.61	30	60

* Experiment 21 shows a decrease in the white corpuscle count.

Experiment 17, November 27, 1917

11.00 a.m. White blood corpuscles 11,600 per cubic millimeter. Red blood corpuscles 6,576,000 per cubic millimeter.

11.05 a.m. 2 cc. secretin solution (representing 20 mgm. of the dried extract) per kilogram of body weight given hypodermatically.

11.35 a.m. White blood corpuscles 13,000 per cubic millimeter. Red blood corpuscles 7,263,000 per cubic millimeter.

12.05 p.m. White blood corpuscles 12,000 per cubic millimeter. Red blood corpuscles 6,956,000 per cubic millimeter.

12.35 p.m. White blood corpuscles 12,600 per cubic millimeter. Red blood corpuscles 6,118,000 per cubic millimeter.

1.05 p.m. White blood corpuscles 11,500 per cubic millimeter. Red blood corpuscles 6,288,000 per cubic millimeter.

3.05 p.m. White blood corpuscles 8,500 per cubic millimeter. Red blood corpuscles 6,052,000 per cubic millimeter.

A dose of 1 cc. of secretin solution per kilogram of body weight produces an average increase of 44.2 per cent in the number of white corpuscles in 51 minutes, while 0.5 cc. of secretin solution per kilogram produces an increase of only 25.42 per cent in 60 minutes and 2 cc. of secretin solution per kilogram an increase of 38.21 per cent in 40 min-

utes. Therefore the conclusion is justified that 1 cc. of secretin solution per kilogram of body weight is the most efficient dose to increase the number of both white and red corpuscles.

In our previous work on the red blood corpuscles we also found that by repeating the dose of secretin solution at short intervals the increase in the erythrocyte count could be kept up for several hours but dropped promptly after the administration of the last dose. In table 4 the results of four experiments are recorded in each of which 1 cc. of secretin solution per kilogram of body weight was injected subcutaneously at hourly intervals for three doses.

It will be seen that the increase in the white corpuscle count produced by the first dose is partly maintained by the succeeding doses but rises after the administration of the last dose so that at the end of five hours the number of white corpuscles in the blood stream is very decidedly greater than at the beginning of the experiment. In the same table are given the erythrocyte counts in the same experiments which confirm the results that we obtained previously. A comparison of the effects produced when repeated doses of secretin are given at short intervals shows that the total effect on the white corpuscles is more marked and more persistent than is the effect on the erythrocytes.

A similar comparison of the effect of a single dose of secretin solution on the red and white blood corpuscles shows the same relation. In practically all cases the effect on the white corpuscles appears as quickly, or more quickly, than the effect on the erythrocytes and persists for a longer time. Also the average percentage increase in the white corpuscle count per unit volume of blood is in all cases nearly or quite double the percentage increase in the erythrocyte count.

In our previous paper we suggested as the most probable explanation of the increase in the number of erythrocytes in the circulating blood produced by secretin that it is due to a direct stimulating action of the secretin on the red marrow of the bones. We are still inclined to believe that this is the true explanation and further work is being done in an endeavor to determine this. It is conceded that there are two sources for the white blood corpuscles, the bone marrow and the lymphatic tissues in general. It would seem probable because of the much greater effect of secretin on the number of the white corpuscles that it stimulates their production by both the bone marrow and the lymphatic tissues. Further work is also being done along this line.

TABLE 4

Dose: 1 cc. secretin solution per kilogram of body weight at hourly intervals for three doses

EXPERIMENT NUMBER		INITIAL COUNT	FIRST HOUR	SECOND HOUR	THIRD HOUR	FIFTH HOUR	SIXTH HOUR	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN
											hours
15	W. B. C.	11,000	13,300	11,600	11,400	14,600	11,800	14,600	3,600	32.72	5
	R. B. C.	4,677,000	4,896,000	5,867,000	5,696,000	5,343,000	5,100,000	5,867,000	1,190,000	25.44	2
16	W. B. C.	9,200	15,300	13,200	12,600	16,200	12,200	16,200	7,000	76.08	5
	R. B. C.	5,280,000	6,608,000	6,768,000	7,160,000	6,076,000	4,708,000	7,160,000	1,880,000	35.60	3
19	W. B. C.	6,800	7,400	7,000	6,800	8,100	7,000	8,100	1,300	19.11	5
	R. B. C.	7,584,000	7,968,000	6,408,000	8,176,000	8,128,000	7,026,000	8,176,000	592,000	7.80	3
20	W. B. C.	6,400	8,800	8,000	6,400	7,800	5,800	8,800	2,400	37.5	1
	R. B. C.	6,288,000	6,672,000	6,608,000	7,488,000	6,886,000	5,876,000	7,488,000	1,200,000	19.08	3
Averages	W. B. C.	8,350	11,200	9,950	9,300	11,675	9,200	11,925	3,575	42.81	4
	R. B. C.	5,957,250	6,536,000	6,412,750	7,130,000	6,603,250	5,677,500	7,172,750	1,215,500	20.40	2¾

CONCLUSIONS

1. It is possible to produce an increase in the number of white corpuscles per cubic millimeter o blood by the administration of secretin, even in small doses and by subcutaneous injection.

2. The most efficient dose is 1 cc. of secretin solution per ki'ogram of body weight.

3. The increase in the count appears quickly and is very transient, but is greater and more persistent than the increase in the erythrocyte count produced by the same means.

4. By repeating the dose of secretin solution at short intervals the increase in the number of both the erythrocytes and the white corpuscles can be kept up for several hours but is more marked and persists somewhat longer after the last dose in the case of the white corpuscles than in the case of the red corpuscles.

5. It is suggested that the effects described are due to a direct stimulating action of secretin on both the bone marrow and the lymphatic tissues in general.

6. The results of previous experiments on the number of erythrocytes in the circulating blood are confirmed.

BIBLIOGRAPHY

(1) Downs and Eddy: This Journal, 1917, xliii, 415.
(2) Lamson: Journ. Pharm. Exper. Therap., 1915, vii, 169; 1916, viii, 167.

VI. FURTHER STUDIES ON THE EFFECT OF ADRENALIN UPON THE BLOOD FLOW IN MUSCLES

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Received for publication December 20, 1917

In 1895 Oliver and Schäfer (1) demonstrated that adrenal extract has a pressor action upon the vasomotor system in both frogs and dogs. In the dogs they plethysmographed several organs simultaneously. These did not of necessity respond in the same manner, at times one contracted and the others expanded and vice versa, and sometimes all the organs under observation expanded. This increase they attributed to a passive expansion due to swelling of the large blood vessels in the limbs.

It has been shown that adrenalin injected into animals (cats and dogs) in small doses (0.1 to 0.5 cc. of a 1: 100,000 solution) produces a fall in blood pressure. This fall Cannon and Lyman (2) say results not only from a lessened peripheral resistance due to vasodilation in the cutaneous system but also to dilation in the splanchnic vessels. Plethysmographic records taken simultaneously with blood pressure records showed that the limb volume increases as the blood pressure falls. Dale (3) came to the same conclusion. He advanced the theory that small amounts of adrenalin stimulate vasodilator endings while large quantities bring the vasoconstrictors into play.

Hartman (4) does not share absolutely in this belief, but believes that adrenalin in small doses is selective in its action. He found that if the limb vessels were clamped adrenalin produced a rise in blood pressure, and if the vessels of the splanchnic area were clamped and the limb vessels left intact adrenalin produced a fall in pressure. He says that toneless relaxed muscles probably do not relax further by injection of adrenalin. Recently Hartmen and Fraser (5) advanced the

[1] A number of the earlier experiments were performed in the Laboratory of Physiology in the Albany Medical College.

theory that the vasodilator action of adrenalin in muscle vessels does not depend upon the tonicity of the vessel wall but upon a center in the central nervous system elsewhere than in the cerebral hemispheres. They obtained dilation in the perfused limbs of dogs upon injecting adrenalin into the circulation when the nervous connections were intact.

Hoskins, Gunning and Berry (6) pointed out that adrenalin injected intravenously in small doses dilates the muscle vessels and constricts the cutaneous. Gruber (7) was unable to obtain vasodilation in muscles the nerves of which had just been cut. His results were confirmed by Hartman and Fraser (5). Nor was he able to obtain dilation in active muscles whose nerves were cut and stimulated at a rate favorable to dilation.

The present research was carried out to determine whether or not adrenalin acted centrally or peripherally in producing vasodilation in muscles.

METHOD

Cats, anaesthetized with ether, were used. The blood pressure was registered by a mercury manometer from a cannula in the carotid artery. A time marker was placed at the atmospheric pressure line of the manometer.

Usually adrenalin chloride, but in some cases crystalline adrenalin (epinephrin) (0.5 to 2 cc. of a 1: 100,000 solution) and in some cases (0.5 to 1 cc. of a 1: 10,000 solution), was injected into a cannula placed in the external jugular vein. In many experiments hirudin (10 mgm. per kilo) was injected intravenously as an anticoagulant.

In all cases the cutaneous vessels were ligated and cut and either the skin removed or the limb mass ligated above the hock. Both methods satisfactorily separate the skin circulation from the muscle circulation. A paraffined glass cannula of small bore (3 mm. diameter) was placed in the femoral vein, all the branches to which were tied off except the deep anterior tibial vein wh[illegible] comes from the tibialis anticus muscle and other muscles of that [illegible]ion. The drops of blood flowing through it were recorded on the [illegible]noked dru[illegible] surface by a signal magnet operated by an automatic circuit breaker (7).

Experiments were performed on several animals in which either the sciatic nerve or the peroneal nerves in the left limb had been cut 2 to 10 days earlier. As many animals were used in experiments on the normal limb, on the limb in which the nerve was cut just previous to

the experiment and on perfused limbs. In these last cases the fluid was forced through a cannula placed in the femoral artery.

RESULTS

Adrenalin injected into a cat in small doses (0.5 to 2 cc. 1: 100,000 solution) brings about a fall in arterial pressure and produces an active

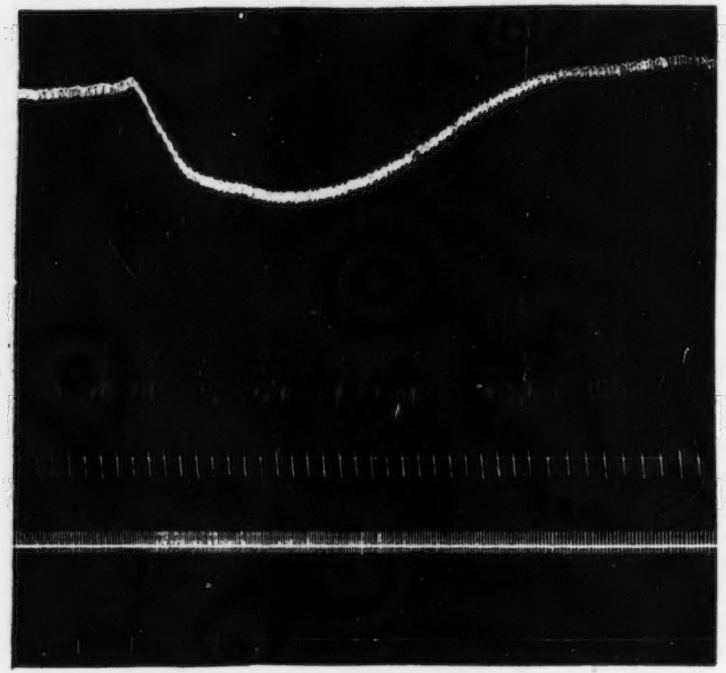

Fig. 1. In this and the following records the upper curve records blood pressure with mercury manometer, below it the time marker and zero blood pressure. Except in Fig. 2 the third line is that of blood flow through the muscle in drops. Unless otherwise stated the time is in 5 second intervals. Hirudin used as an anticoagulant. Nerve intact, time in 15 seconds.

vasodilation of the muscle vessels (see fig. 1). The same dose of adrenalin was always found to produce vasoconstriction of the skin vessels. These results corroborate those obtained by Hoskins, Gunning and Berry on dogs and Hartman and Fraser on cats with the

plethysmograph. The animal from which this figure was taken weighed 2 kilos and received 20 mgm. of hirudin intravenously. At the point indicated in the record 0.5 cc. of adrenalin (1: 100,000 solution) was injected. There resulted a fall in blood pressure from 110 to 80 mm. of mercury or 32 per cent, and an increase in blood flow through the muscle vessels from 32 to 48 drops per 30 seconds.

Fig. 2. Curve showing the effects of cutting the nerve upon the muscle circulation. Nerve cut at *1*.

In many instances adrenalin in larger doses produced a rise in arterial pressure with the muscle vascular system still showing a dilation. In order to compare the blood pressure in the limb with that of the general circulation two mercury manometers were employed, one in the carotid and the other in the femoral artery of the limb not under investigation. By looping a ligature around the aorta just above the iliac axis it was possible to control the blood pressure in the vessels

of the limb under investigation. Even though the blood pressure did not increase in this limb, there was a marked increase in blood flow through the muscle.

Figure 2 is given to show the effect of cutting the nerve to the muscle. For the 10 seconds preceding the cutting of the nerve at *1* the rate of

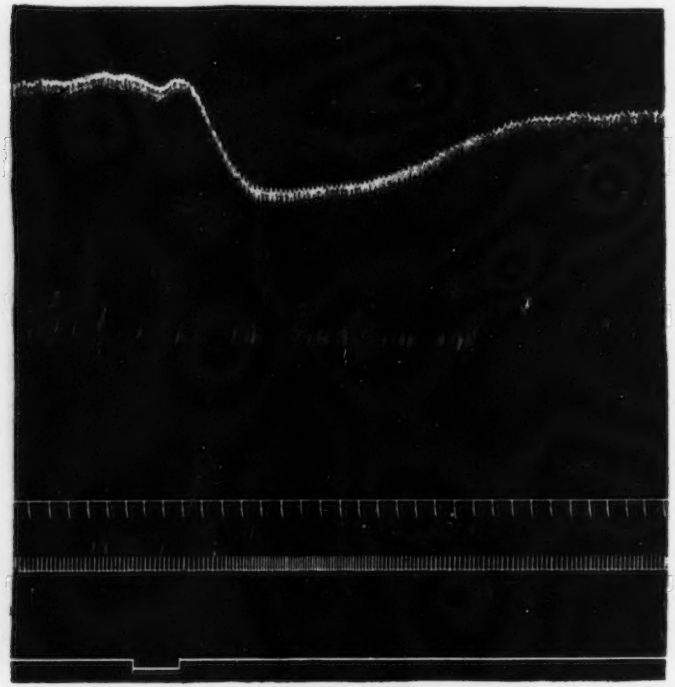

Fig. 3. Peroneal nerve cut 4 days and the sciatic cut previous to this record; 0.5 cc. of 1: 100,000 adrenalin.

blood flow through the vessel was 16 drops. After cutting, the rate of flow was increased to 47. In all cases it was noted that within 15 to 30 seconds after the nerve was cut the rate of blood flow through the muscle was from 2 to 3 times faster than before. This is of course due to loss of tonicity in the vessel wall.

There is in muscles under such experimental conditions no increase

in flow upon injection of adrenalin unless there is considerable rise in blood pressure during which a slight passive dilation may be recorded. This confirms Gunning's (8) work with large doses of adrenalin. If the pressure falls there is either a passive constriction due to the fall in the blood pressure, especially when the pressure is low, or an active vasoconstriction due to the action of the adrenalin itself. Hartman suggests that, since "a depressant substance obtained from ox pituitaries" caused further dilation in the denervated limbs, the inability of adrenalin to bring about further dilation under similar conditions is not due to the absence of muscular tonicity but to the loss of connection with a vasodilator center. It might be questioned in this connection whether or not the points of action of adrenalin and the depressant substance from ox pituitaries are the same.

It is my belief that this lack of vasodilator action of small doses of adrenalin in denervated limbs is due to the loss of tonicity; that when the tonicity of the vessel wall is normal adrenalin causes vasodilation and when the tonicity is lost it causes constriction. In both cases it acts upon the peripheral vasomotor mechanism. To substantiate this theory figures 3 and 4 are presented.

The effect of nerve degeneration (2 to 10 days). It is a well known fact that if time is allowed for recovery after cutting the nerves to vessels a certain amount of tonicity of the vessel wall is regained (9). Bowditch and Warren (10) demonstrated that the vasoconstrictor nerve fibers are the first to degenerate after section of a mixed nerve. In this present research some experiments were performed upon animals in which either the peroneal or the sciatic nerves were cut from 2 to 10 days previous to the experiments. Somewhat variable results were obtained but this variation was due to the degrees of inflammation in the sectioned limbs. A dilation was never observed in limbs in which there existed marked inflammation and edema. In eleven animals in which healing took place without infection, the vessels responded with dilation to adrenalin as well as did the vessels of normal uninjured muscles.

Figures 3 and 4 are records obtained from animals in which the nerves had been cut 4 and 6 days, respectively, previous to the experiments. In the former the peroneal, in the latter the sciatic nerve was sectioned. In order to make sure that the vasomotor center was inoperative on the area drained by the venous cannula the sciatic nerve was cut just before figure 3 was made. As a result no change in blood flow was noted. At the point indicated in the record 0.5 cc.

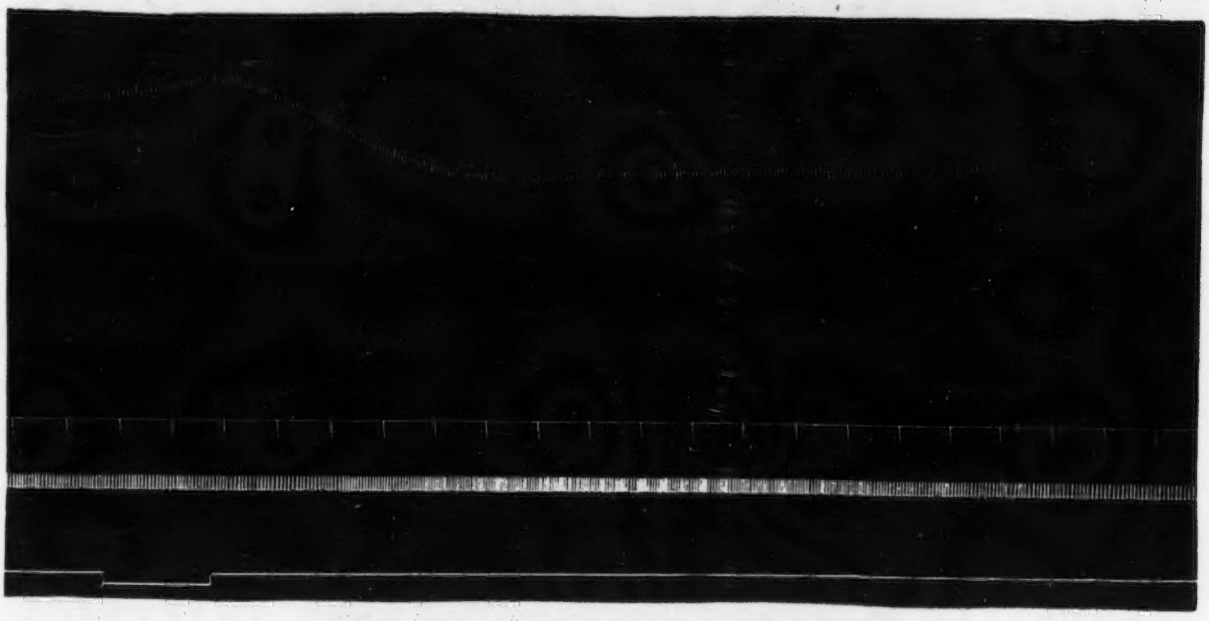

Fig. 4. Sciatic nerve cut 6 days; 0.5 cc. of 1: 100,000 adrenalin.

adrenalin (1: 100,000) was injected intravenously. There resulted a fall in blood pressure from 124 to 90 mm. of mercury with a resultant increase in flow of blood through the muscle from 25 to 43 drops per 30 seconds. In figure 4, 0.5 cc. of adrenalin (1: 100,000) slowly injected intravenously caused a fall of blood pressure from 98 to 74 mm. of mercury and an increase in the rate of blood flow from 90 to 150 drops per 30 seconds.

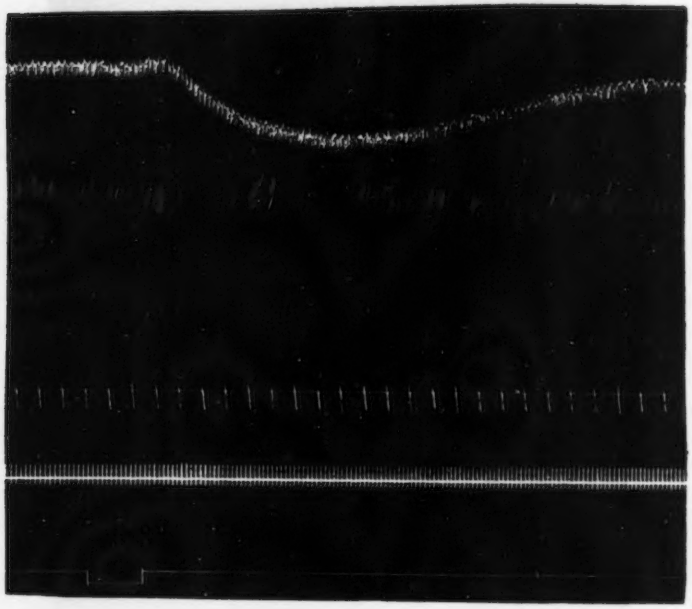

Fig. 5. Perfused limb with nerve intact; 0.5 cc. of 1: 100,000 adrenalin.

In two animals with denervated limbs all the muscles were severed at the thigh leaving only the artery intact and the femur to support the anterior tibial muscle and other muscles of the lower leg. In both instances when there could be no possible connection with a vasodilator center there resulted from injections of 0.5 cc. of adrenalin (1:100,000) the usual vasodilation.

In the animals in which the nerves were cut some time previous to

the experiment an increase in the blood flow was obtained even with a blood pressure as low as 30 mm. of mercury. It was observed that a larger dose of adrenalin was necessary to produce vasoconstriction.

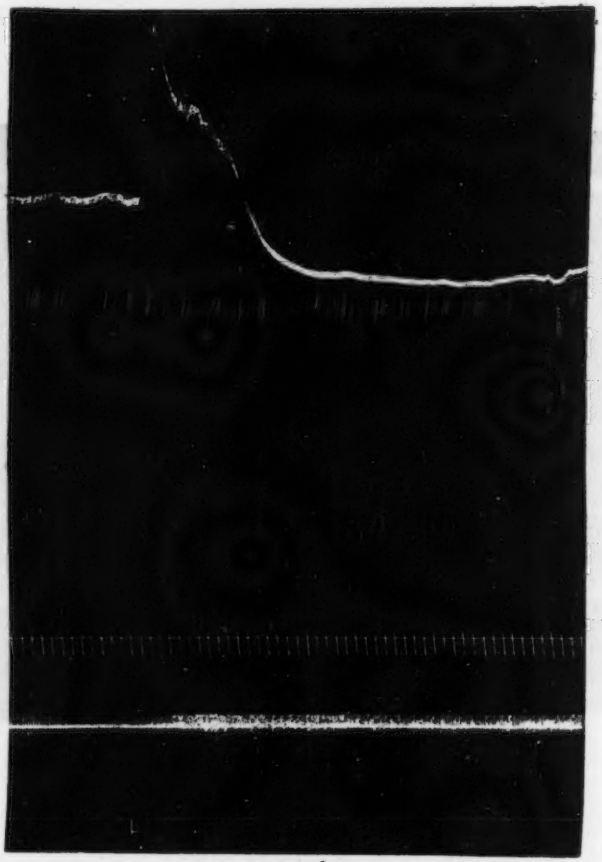

Fig. 6. Perfused limb with nerve intact; 0.5 cc. of 1: 10,000 adrenalin.

In one animal with nerve cut 6 days, 3 cc. adrenalin (1: 100,000) rapidly injected was necessary to produce vasoconstriction, whereas 0.1 to 0.5 cc. (1: 100,000) produced the same result in the limb in which the nerve had just been severed.

Perfusion experiments. Eleven experiments were performed upon animals in which the limb was perfused with oxygenated Ringer's solution or oxygenated defibrinated blood at a constant temperature. This varied in the different animals from 37° to 38.5°C. but was constant for each experiment. The pressure for the perfusion fluid was 90 cm. of water. The flask containing the perfusion fluid was placed within a container filled with water at a constant temperature of 38.5°C. The fluid then flowed into a 100 cc. flask placed within another container through which a stream of water at 38.5°C. ran to maintain a constant temperature within the perfusion flask. In order to make sure that the temperature was constant a thermometer was placed in the perfusion fluid just before it entered the cannula.

The limb was first ligated above the hock. The femoral vein and artery were isolated without injuring the nerves, as in my previous experiments, and ligatures were looped loosely around them to make ready to insert the cannulae.

The abdominal aorta was isolated and a ligature placed loosely around it. The venous cannula was then inserted and the limb tested for normal vasodilation. The artery was quickly clamped, a cannula inserted and the perfusion started, after which the loop around the aorta was tightened. This reduced the manipulation of the tissues between the normal test and the perfusion test to a minimum. In a few experiments one limb was perfused and the other limb was bled from the venous cannula. In all but two cases a fall in blood pressure was obtained by injecting 0.5 cc. 1: 100,000 solution of adrenalin intravenously. In two animals this amount gave a rise in blood pressure followed by a fall. In every instance it produced a dilation of the normal intact limb (fig. 1) but no change in the perfused limb with the nerve intact (fig. 5). That this difference in reaction cannot be due to injury through manipulation of the vessels is shown by the fact that the normal limb still acts by vasodilation to small doses of adrenalin and that a fall in blood pressure is still obtained. Moreover the only injury caused after the operations on the normal limb is that of placing a cannula in the previously isolated femoral artery and the tying of the ligature already looped around the aorta. If adrenalin exerted its influence entirely through a vasodilator center it should produce the same results in these two cases where the only difference in the conditions of the limbs is that one has and one has not the circulation intact.

Dilation of the perfused limb was recorded in cases where much

stronger solutions (0.5 to 1 cc. 1: 10,000 solution) were used. In every case the blood pressure was markedly increased (fig. 6). These results corroborate Hartman and Fraser's results for they present a similar rise in blood pressure and active vasodilation.

My results with small doses of adrenalin (0.5 cc. 1: 100,000 solution) support Cannon and Lyman's view and Dale's theory based upon experiments with ergotoxin and adrenalin showing that adrenalin acts peripherally rather than centrally. With large doses of adrenalin (0.5 to 2 cc. 1: 10,000 solution) active vasodilation is observed in perfused limbs, which fact supports Hartman and Fraser's theory that adrenalin excites a vasodilator center. It may be, however, that this dilation is due to depression of the vasoconstrictor center through increased blood pressure (9). Pilcher and Sollman's (11) experiments render this latter view untenable.

SUMMARY

Adrenalin in small doses (0.5 to 2 cc. 1: 100,000 solution) produces active dilation of the vessels in cats' muscles when the nerves are intact. These results coincide with those obtained by Hoskins, Gunning and Berry experimenting on dogs and Hartman and Fraser on cats.

In my experiments, adrenalin, in any strength, produced no active vasodilation in muscles in which the nerves were recently cut.

Small doses of adrenalin (1: 100,000) slowly injected intravenously produce active vasodilation in muscles in which the nerves were previously cut and allowed to degenerate from 2 to 10 days. This renewal of the action of adrenalin may be due to a partial recovery of the tonicity of the vessel walls.

Small amounts of adrenalin (0.5 cc. 1: 100,000) injected intravenously did not produce vasodilation in the eleven experiments in the perfused limb intact with the central nervous system. A fall in blood pressure was obtained in every case showing dilation somewhere in the vascular system.

In perfused limbs large amounts of adrenalin (0.5 cc. 1: 10,000) produced active vasodilation accompanied by a rapid increase in blood pressure followed by a fall. Hartman and Fraser attribute this dilation to excitation of a vasodilator center. Further work is necessary to prove conclusively whether or not this vasodilation is due to depression of the vasoconstrictor center by the increased blood pressure or to excitation of a vasodilator center by the adrenalin.

The vasodilation in muscles caused by small amounts of adrenalin is dependent upon the tonicity of the vessel wall.

Small doses of adrenalin (0.5 cc. 1: 100,000) bring about vasodilation by their action on the peripheral vasodilator mechanism.

BIBLIOGRAPHY

(1) Oliver and Schäfer: Journ. Physiol., 1895, xviii, 233.
(2) Cannon and Lyman: This Journal, 1913, xxi, 384.
(3) Dale: Journ. Physiol., 1905, xxxii, 59; Ibid: 1906, xxxiv, 169.
(4) Hartman: This Journal, 1915, xxxviii, 439 and 444.
(5) Hartman and Fraser: This Journal, 1917, xliv, 353.
(6) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(7) Gruber: This Journal, 1917, xliii, 530.
(8) Gunning: This Journal, 1917, xliii, 395.
(9) Schäfer: Textbook of physiology, London, 1900, II, 136.
(10) Bowditch and Warren: Journ. Physiol., 1886, vii, 416.
(11) Pilcher and Sollman: Journ. Pharm. Exper. Therap., 1914, vi, 341.